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CONTENTS

Intermittent Growth of Fruits of Phalaenopsis. A Correlation of the Growth Phases of an Orchid Fruit with Internal Development. *Robert E. Duncan and John T. Curtis* 167

Vitamin Deficiencies of Ceratostomella. *William J. Robbins and Roberta Ma* 184

Effect of Variations in Light Intensity, Length of Photo-Period, and Availability of Nitrogen upon Accumulation of Ascorbic Acid in Cowpea Plants ..... *Mary Elizabeth Reid* 204

The Relation between Xylem Thickenings in Primary Roots of Vicia faba Seedlings and Elongation, as Shown by Soft X-Ray Irradiation ..... *G. F. Smith and H. Kersten* 221

Somatic Chromosomes of Aconitum noveboracense and A. uncinatum. *Dorothy J. Longacre* 235

Studies in the Ericales: A New Name in Blueberries..... *W. H. Camp* 240

A New Campanula from Idaho ..... *Rogers McVaugh* 241

New Combinations and New Names in the Umbelliferae—II. *Mildred E. Mathias and Lincoln Constance* 244

Index to American Botanical Literature 249

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BULLETIN OF THE TORREY BOTANICAL CLUB
69(3): 167–183 MARCH, 1942

INTERMITTENT GROWTH OF FRUITS OF PHALAENOPSIS. A CORRELATION OF THE GROWTH PHASES OF AN ORCHID FRUIT WITH INTERNAL DEVELOPMENT

ROBERT E. DUNCAN AND JOHN T. CURTIS[1]

It has been recognized that the orchid ovary is not mature at the time of pollination since the work of Brogniart (1831) and confirmation of his findings by a number of mid-nineteenth century botanists. Hildebrand (1863), Treub (1883), and Guignard (1886) determined the periods of time which elapse between pollination, maturation of the macrogametophytes, fertilization, and appearance of the embryos, respectively, for a considerable number of species. Veitch (1888) extended this type of observation to *Cattleya labiata* var. *mossiae*. In general, the periods of time between the phases of the reproductive process vary greatly from genus to genus and from species to species within the same genus. For example, Hildebrand (1863) found that 110 days after pollination the ovaries of *Dendrobium nobile* contained ovules possessing immature macrogametophytes and both integuments, and that 130 days after pollination embryos were developing. In *Orchis mascula* the respective periods were 19 and 28 days; in *O. pyramidalis* 7 and 10 days.

Brown (1833) commented that the first pollen effect is the enlargement of the ovary and ovules. Crüger (1851) observed that pollination had a dynamic effect on fruit development in *Vanda* in that ovules are only formed after pollination. This effect, he believed, took place before the entrance of the pollen tubes into the ovary cavity, in contrast to the effect brought about by the material contribution of the pollen tubes at the time of fertilization. Hildebrand (1863) clearly recognized the "double effect" of pollination which he said was, first, the increase in size of the ovary and the maturation of the ovules, and second, fertilization. He recognized that unpollinated flowers fell off the plant, even though the ovules in some cases grew to a limited extent. Hildebrand (1865) found that in many cases foreign pollen induced fruit-set. He considered that a green enlarging ovary was of primary importance to the development of the ovules.

The work of Fitting (1909) and Laibach and Maschmann (1933) demonstrated by the behavior of the column that the pollinia contain a growth-regulatory substance. In general they studied an immediate pollination reaction characterized by the growth of the edges of the stigmatic cavity in

[1] Part of the cost of illustrations for this paper was borne by the Lucien M. Underwood Memorial Fund.

[THE BULLETIN for February (69: 75–166) was issued February 2, 1942]

such a fashion as to enclose the pollinia.[2] Guignard (1886) suggested that the enlargement of the ovary accompanied the growth of the pollen tubes to the base of the ovary.

That pollination is necessary for fruit-set and in some indirect manner for ovule development is clearly demonstrated by Hurst's (1898) report of matroclinous progeny resulting from attempted crosses between *Zygopetalum mackayi* and various species of *Odontoglossum*, *Oncidium*, and *Lycaste*, respectively. Suessenguth (1923) has shown that embryos formed in such crosses are of apomictic origin. They are not formed, however, unless pollination takes place.

Phalaenopsis, the moth orchid, a Malasian member of the *Sarcanthinae*, grows as an epiphyte in the rain forests. Like other orchids the fruit is a three-valved capsule containing innumerable minute seeds. In the United States and Europe it is frequently used as a source of cut flowers, for under good growing conditions a plant with 10–12 basal leaves may produce a laterally branched inflorescence with as many as 170 flowers. The genus has seldom been used as a subject of cytological, morphological, or physiological research. Palm (1920) reported that cytokinesis is simultaneous after meiosis in pollen mother cells. Treub (1879) recorded the presence of haustorium-like suspensors in the embryos of *Phalaenopsis grandiflora*, *P. schilleriana*, and *P. spectabile*. In a series of papers beginning with that of Fitting (1909) and culminating with that of Laibach and Maschmann (1933) *Phalaenopsis* was one of the orchids in whose pollinia a growth regulatory substance was demonstrated and studied.

Burgeff (1934), using seeds of 11 species and hybrids of *Phalaenopsis*, reported that the great majority of them gave germination percentages of less than 10 per cent when grown on "vitamin-free" media, while the addition of an unidentified vitamin or growth substance isolated from yeast and certain soil fungi increased the germination to 90 or 100 per cent. On the other hand seeds of *Phalaenopsis sanderiana* and *P. equestris* germinated 100 per cent without an external source of growth substance.

METHODS AND MATERIALS

The various individual studies reported herein were made in winter and

[2] Our attention has been called to a recent paper on the effects of synthetic growth-regulatory compound on the column in bringing about several postfloral phenomena (**Hubert, B., & Maton, J.** 1939. The influence of synthetic growth controlling substances and other chemicals on postfloral phenomena in tropical orchids. Biol. Jaarb. **6**: 244–285). The same authors produced parthenocarpic fruits in a *Cymbidium* hybrid and in *Oncidium longipes* by the application of pure crystals of naphthaleneacetic acid on the stigmas (Parthenocarpie en groeistof. Natuurwetenschap. Tijdschr. **21**: 339–348. 1940). These fruits reached a considerable size. Unfortunately we have never seen the original papers and have only the information derived from abstracts.

early spring of subsequent years in the botany greenhouses of the University of Wisconsin and the conservatory of Dr. C. K. Schubert, both in Madison, Wisconsin, and in the greenhouses of the Morris Arboretum and the Botany Department of the University of Pennsylvania, both in Philadelphia.

In these studies plants of *Phalaenopsis pamala*, *P. fontainebleau*, *P. aphrodite*, and *P. schilleriana* were used. Of these the latter two are naturally occurring forms; *P. fontainebleau* is a hybrid between *P. wiganiae* (a primary hybrid between the species *P. schilleriana* and *P. stuartiana*) and *P. schilleriana*. *Phalaenopsis pamala* is a hybrid between *P. fontainebleau* and *P. elizabethiae* (a primary hybrid between the species *P. amabilis* and *P. rimstadtiana*).

In any one study the amount of pollen applied to an individual stigmatic surface was macroscopically similar. It consisted of one or two pollinia, the latter being the probable number in natural pollination since each pair of pollinia derived from an anther is connected to an adhesive disc. The pollinations were self, close, or reciprocal. Flowers of approximately the same age, on various branches of the same inflorescence, were pollinated or used as pollen source in close pollinations. In reciprocal pollination the particular pollinium or pair of pollinia applied to any one flower was selected at random from the lot of pollinia derived from the flowers to be pollinated on the other plant. Of the total number to be used for study an approximately equal number of flowers on each branch of an inflorescence was pollinated.

The diameter of the developing fruit was measured to 0.1 mm. with vernier calipers; the length with vernier calipers or a flexible millimeter rule. These measurements were made possible, although the ovary is inferior, by the fact that immediately below the level of sepal insertion the fused structure, representing ovary and receptacle, is lined with six grooves which terminate uniformly in the flower and early stages of ovary maturation. Freehand sections show that the base of the ovary cavity is closely associated with the groove ends. As the development of the ovary proceeds the groove lengths of an individual ovary may vary slightly from the bottom to the top sides, the top grooves generally being shorter. This difficulty was met in different studies either by taking an average of the length of top and bottom grooves at each measurement, beginning at the time when the discrepancy appeared, or by disregarding all but one groove which then became the index of ovary growth in length. In occasional flowers the association of groove ends with the termination of the ovary swelling is varied by the grooves stopping slightly short of the swelling or passing beyond it down the pedicel. These variations may be noted on different ovaries of the same plant. Such phenomena were disregarded and the groove measurements were used. Since the ovary tapers from the level of perianth insertion

to the pedicel during the earlier stages of development, a slight distance above or below the approximate middle will give larger or smaller diameters respectively. Small cuticular scars, where the last measurement was made, gave reasonable certainty that the diameter measurements were taken at the same level. In later studies, ovaries were marked with India ink at the approximate center and at both proximal and distal ends. In this way measurements at the same level were assured each time.

In preliminary studies a number of flowers were pollinated and, at set periods of time, one of the ovaries was measured. The ovary concerned was removed, weighed, and fixed in Randolph's modification of Nawaschin's fixing solution for use in determining the state of ovule development and the fruit wall changes. Entering the measurements obtained from subsequent fruits and joining the points of length and of diameter respectively resulted in two growth curves. Such curves are open to criticism on the permissibility of connecting points so derived. Such a study, however, was necessary to locate the various internal changes with respect to growth phases.

In a second series of studies a number of flowers on a single plant were pollinated. The group was sampled by removing at random a fruit for determination of the state of internal development and for weighing. The points of the growth curves for length and diameter were determined by the average of a smaller and smaller number of developing ovaries as the study progressed. A growth curve erected on such data can be criticized on the basis that the larger share of food, available to the remaining young fruits after removal of one, might falsify the slope of the curve immediately following the removal.

In a third series of studies all the fruits were allowed to develop to maturity. While these studies did not disclose fruit wall or ovular changes they provide a series of continuous measurements of individual fruits free from the effect of shifting food supply except as may occur naturally. (The illustrations present graphically the development of representative fruits from this series of studies.)

After the fruits had ripened, examinations were made to determine the percentage of seeds containing fully formed embryos. The seeds were usually sharply divided into two groups: those containing apparently normal embryos and those consisting only of empty seed coats. As a further test, samples of seeds from each of the pods were sown on a medium previously found suitable for the germination of a variety of orchid seeds (Curtis 1936), modified by addition of the necessary complement of vitamins. These cultures were maintained under constant environmental conditions (temperature, 30° C.; relative humidity, 80 per cent; light, 20 hours per day at

150 f. c.). Germination is reported in the legend of the graphs as percentage of seeds which originally contained embryos, disregarding those which were empty.

RESULTS

Within 24 hours after pollination the petals and sepals are noticeably wilted; within 48 hours the turgidity is lowered to the extent that the venation of petals and sepals is distinct. At the close of this period the petals have folded forward over the column and the upper sepal has bent forward from its former reflexed position. These perianth changes are concomitant with growth of the column in width and thickness and with growth of the edges of the stigma over the cavity in which the pollinia become imprisoned. The columnar growth continues for 9 or 10 days, the major part taking place during the first few days. The column remains swollen during much of the early period of fruit maturation; later it dries down considerably.

The first response of the ovary to pollination is a slight increase in its curvature immediately below the insertion of the perianth. This curvature is soon lost in the general growth of ovary in length and diameter. The accompanying graphs (figs. 1, 2, 3) illustrate the extent and general rate of growth and the fact that the curves for growth in both diameter and length are not simple sigmoid ones. Comparison of the growth curves of *Phalaenopsis fontainebleau* (fig. 1), *P. schilleriana* (fig. 2), and *P. pamala* (fig. 3) shows the same general configuration to be present although there are minor differences in the total extent and the time and duration of the maximum rate of growth. Figure 4 shows the relationship which the increase in fresh weight of the ovary of *P. fontainebleau* bears to the increase in calculated volume of the ovary.

Cross sections of the ovary of the unpollinated flower show the wall, slightly triangular in outline, to be made up of six lobes each with a vascular supply. Three lobes occupy the corners of the triangle. The three lying between each pair of these bear the placentae, long ridges inserted opposite the vascular supply throughout the length of the ovary. In cross section the placentae appear somewhat anchor-shaped and closely fit together, leaving little space in the central part of the ovary.

Within two days after pollination nuclear and cell divisions can be found in cells of the inner faces of the three sterile lobes and in the cells constituting the margin of the placentae. The cell divisions in the placentae have no particular orientation, the result being a general increase in the shoulders. This building up of the flanges or shoulders leads to an inversion of the placental shape from a ridge with reflexed margins to a troughed structure with flanges jutting above the attached median portion. The fact that the two flanges may reach a greater length than the attached portion eventu-

FIG. 1

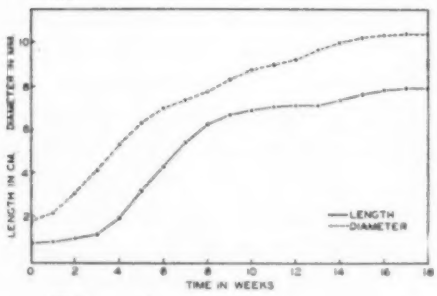


FIG. 2

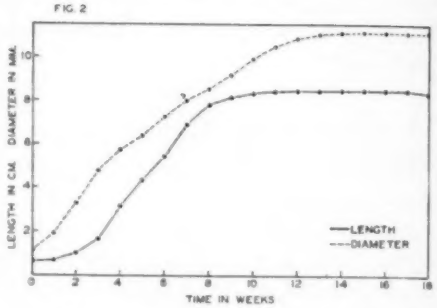


FIG. 3

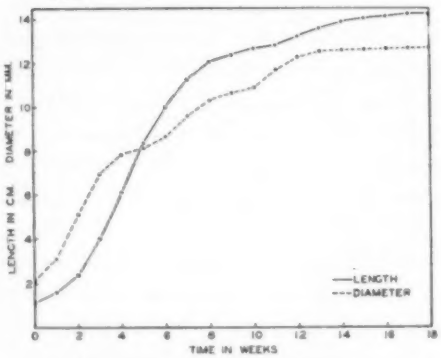


FIG. 4

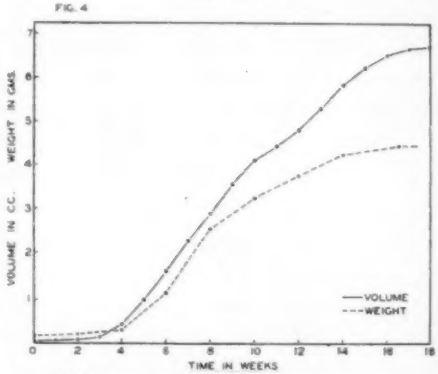


FIG. 5

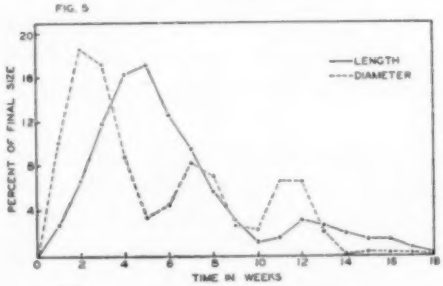


FIG. 6

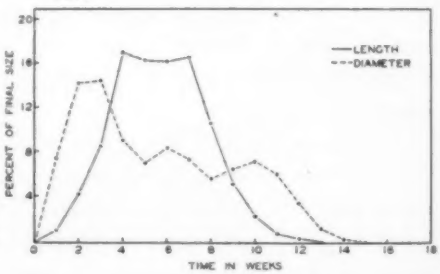


FIG. 7

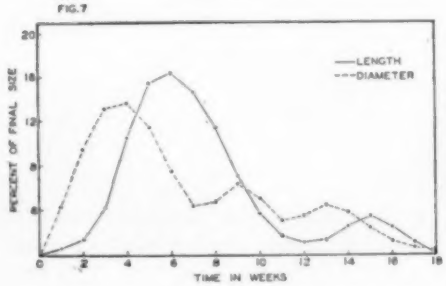
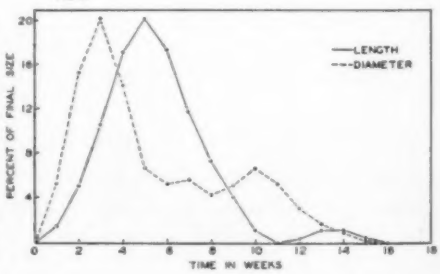


FIG. 8



ally leads to undulations of the flanges. The flanges are not necessarily continuous along the length of the placentae but may consist of a number of flat plate-like structures.

Nuclear and cell divisions in the cells of the ovary wall layers toward the cavity are mainly periclinal, although a few take place along the longitudinal axis of the ovary. The periclinal division and subsequent periclinal growth of the three or four inner cell rows and the enlargement of the outer cell layers allow an increase in the ovary cavity. While it seems probable that there would be a number of nuclear and cell divisions taking place along the longitudinal axis to provide for growth in length of the ovary, aside from the divisions just mentioned and another series involving the epidermal and hypodermal cell layers about 2 or 3 weeks after pollination, no other areas in which cell divisions were taking place have been noted. The cells making up the ovary wall of the unpollinated flower are only slightly elongated, the cells of the inner cell layers being less elongated than those of the outer. The growth and maturation of cells already present in the ovary wall layers account for the growth in length of the ovary and to a limited extent for the growth in diameter.

The pollen germinates within the first four days after being placed on the stigma. The tubes grow down the canal of the column until they reach the ovary cavity, where the single group of tubes divides into three strands, one passing to each of the three placentae. Upon reaching the placenta a strand of pollen tubes divides again; each half passes down one side of the placenta. Fourteen days after pollination the longest pollen tubes have reached the base of the ovary cavity, but the majority are still growing in the upper part of the ovary. Two nuclei are present near the tip of the pollen tube. While the pollen tubes are growing down along the placentae, rows of blunt-tipped glistening hairs arise from the inner ridge of the three sterile lobes of the ovary. These hairs increase in length and persist until the fruit is mature. Occasionally in certain crosses the pollen does not germinate or, if it does, the pollen tubes do not penetrate to the ovary cavity.

Explanation of figures 1–8

FIGS. 1–3. Growth curves of individual fruits of *Phalaenopsis*. FIG. 1. *P. fontainebleau* at the Morris Arboretum. FIG. 2. *P. schilleriana* at Dr. C. K. Schubert's conservatory. FIG. 3. *P. pamala* at the University of Wisconsin. FIG. 4. Curves presenting the increase in calculated volume and fresh weight, respectively, of fruits of *P. fontainebleau* at the Morris Arboretum. FIGS. 5–8. Curves presenting the weekly growth increments of individual fruits of *Phalaenopsis*. FIG. 5. *P. pamala* at the University of Wisconsin. (91.0 per cent seeds possessing embryos; 92.6 per cent germination.) FIG. 6. *P. schilleriana* at Dr. C. K. Schubert's conservatory. (93.8 per cent possessing embryos; 95.1 per cent germination.) FIG. 7. *P. fontainebleau* at the Morris Arboretum. (88.5 per cent seeds possessing embryos.) FIG. 8. *P. fontainebleau* at Dr. C. K. Schubert's conservatory. (98.0 per cent seeds possessing embryos; 96.2 per cent germination.)

In *Phalaenopsis pamala* at the botany greenhouses of the University of Wisconsin the placentae proliferate for 3 or 4 weeks; in *P. schilleriana* at the Morris Arboretum this period lasts over 6 weeks. The final branches in either case are small mound-like structures which differentiate during the ensuing 10 to 14 days into an axial row of cells surrounded by a sheath of cells one row thick. The inner integument appears as a collar-like growth at this time and is shortly followed by the initiation of the outer integument. As this latter structure develops the ovule becomes anatropous, the funiculus being fused to one side of the outer integument. By the time the integuments have reached the apex of the nucellus, the terminal cell of the axial row has enlarged to become the macrospore mother cell. In *P. pamala* ovules in this stage of development are present about 5 weeks after pollination or 3 weeks after their initiation; in *P. schilleriana* at the Morris Arboretum the periods are 12 and 6 weeks respectively.

During the course of development of the macrogametophyte the upper part of the ovule elongates and the outer integument grows on beyond the inner so that there is some space between the micropyles of the two integuments. Fertilization takes place in *P. pamala* during the ninth and tenth weeks. Since the ovules are not all in exactly the same stage this period is not sharply defined. With ovules containing two and three-celled embryos may be found a few ovules, at the placental extremities, which are still in the macrospore mother cell stages. Capsules of the *P. schilleriana* series at the Morris Arboretum opened in the fifteenth week without any viable seeds. Two weeks earlier the ovules had become anatropous, the two integuments just growing over the apex of the nucellus.

Shortly after fertilization the pollen tubes disappear. The ovule as a whole elongates markedly. The embryo grows for a period of several weeks and comes to displace all the tissues of the ovule except the outer integument which becomes the sole layer of the seed coat. The embryo of the mature seed fills only a portion of the cavity within the seed coat. Suspensors in *P. pamala* were not observed to grow out through the micropyle into the placenta. The funiculus has no vascular supply.

At the close of the period of fruit maturation the changes are slow. The grooves become deeper; the dark green color fades to gray green; lenticels become evident; and there is a decided increase in the amount of cutin. About six months after pollination the fruits of *P. pamala* become slightly softer and yellowish.

Study of ovary wall changes shows that, while there is a period of accelerated growth followed by a period of slower growth for both placental depth and wall thickness, the two structures come to occupy a smaller percentage of the diameter of the entire fruit. The ovary cavity increased rapidly during three periods of ovary development. This increase in ovary

cavity is illustrated by figure 4 which shows a divergence between the weight and volume of the fruit, this divergence becoming particularly noticeable after the eighth week. *Phalaenopsis pamala*, *P. schilleriana*, and *P. fontainebleau* all in the conservatory of Dr. Schubert, and *P. fontainebleau* in the botany greenhouses of the University of Pennsylvania each had approximately the same periodicity of growth as *P. pamala* in the Wisconsin botany greenhouses.

Table 1 summarizes the morphological changes and presents a general schedule of fruit development.

TABLE 1

Schedule of events in fruit development

Period of time after pollination	Morphological and physiological changes
2 days	Perianth wilted; edges of stigmatic cavity enclose pollinia; nuclear and cell divisions in cells of inner parietal layers of ovary wall and beginning of such divisions in placentae.
4 days	Pollen germinates.
10 days	Columnar growth about finished.
25 days	Placental proliferation decelerating.
35–40 days	Differentiation of ovules.
55–60 days	Maturation of macrogametophytes.
63–70 days	Fertilization; degeneration of pollen tubes.
180–190 days	Fruit matures.

DISCUSSION

Inspection of growth curves for both length and diameter shows that each is made up of several sigmoid parts. Plotting weekly increments of growth against time results in a graph which clearly portrays three phases of growth in diameter and one, sometimes two, in length (figs. 5, 6, 7, 8). The increment of growth for each week is plotted in terms of percentage of the final size reached by that particular fruit. In this way, all graphs are on the same scale and are directly comparable with one another. The plotted increment points are connected by lines for ease in inspection. In *Phalaenopsis pamala* (fig. 5) the first and larger phase of growth in length falls between the maxima of the first two growth phases in diameter at about the time of the minimum rate between the two. In *P. schilleriana* (fig. 6) the first phase of growth in length is bimodal except under unusual conditions, possessing two maxima of approximately equal magnitude. The first of the two maxima has about the same relationship to growth in diameter as does the peak rate of the first phase of growth in length in *P. pamala*. In *P. pamala* (fig. 5) after growth in length has reached its maximum rate and is becoming slower there is a period during the sixth and seventh week of development when the rate of deceleration is halted. This corresponds roughly

to the second maximum in the first phase of growth in length in *P. schilleriana* (fig. 6). In both forms this main growth phase lasts about 10 weeks.

In all the forms studied there are at least three periods of accelerated growth in diameter (figs. 5, 6, 7, 8). When there is a second phase of elongation it is of comparatively small magnitude and is loosely associated with the last phase of accelerated growth in diameter. The fact that data from cross pollination between different plants of the same hybrid (fig. 5), between different hybrids, and between hybrids and species (figs. 6, 7), as well as self and close pollinations of hybrids or species, all yielded growth curves of the same fundamental type emphasizes that this type of growth is the rule, at least in this section of the genus *Phalaenopsis*, and that there must be common morphological and physiological behaviors. The possibility that the type of growth found is a response to chance environmental factors is ruled out by this common behavior and by the fact that studies of all forms except *P. aphrodite* were made under several greenhouse conditions in the same or different years and started at various times of the year. For example, nine studies were made with *P. pamala* over a period of four years in two different greenhouses and with pollination dates varying from October to March, yet all growth curves are essentially alike. (This does not imply that environment will not affect the magnitude or the rate of the phases of ovary development; a comparison of figures 7 and 8 does not bear out such a conclusion.) The duration of any one experiment (the time required for fruit ripening) was approximately six months. The high percentage of seeds set and of seeds which germinated demonstrate that the fruits studied were not abnormal.

In *Phalaenopsis*, as has been pointed out, the first noticeable changes after pollination are wilting of the perianth segments, enclosing of the pollinia by the stigma, an increase of the curvature of the ovary, and lengthening and swelling of the ovary. Within two days the cells forming the ovary wall layers next the central cavity are dividing. This is long before the pollen tubes enter the ovary; actually these changes take place whether or not the pollen germinates. In case the pollen fails to germinate or the pollen tubes fail to enter the ovary cavity, the ovary drops off the inflorescence during the ensuing two weeks by means of an abscission layer at the base of the pedicel. Thus the presence of the pollinia on the stigma is sufficient to produce the first three effects and to initiate ovary growth. All these changes are probably in response to growth-regulatory substances present in the pollinia. The perianth and column changes were the particular object of study by Fitting and his coworkers. The wilting of the perianth takes place at the time of the initiation of column and ovary growth when there is a decided modification of water relations. The initial dose of growth-

regulatory substance derived from the pollinia is insufficient, however, to prevent fruit drop unless there is some further development.

This further development is undoubtedly the entrance of pollen tubes into the ovary. The increment curves of many individual ovaries show an initial lag in growth in length; at the conclusion of this lag there is a sharp increase in rate. It is at this time that the pollen tubes enter the ovary cavity. Pollen tube growth approximately keeps pace with length growth of the ovary. The pollen tubes in the ovary may function by secreting growth-regulatory compounds, by providing a tactile stimulus, or by exerting a mechanical force by reason of their hydrophilous nature.

The second period of elongation falls at a time when the embryos are growing. Its significance is little understood; certainly when present it would function in stretching the placenta, thus somewhat separating the seeds. It could be caused by growth-regulatory substances produced within the embryo. These substances may pass through the suspensor which, however, has not been observed to grow into the placenta in the forms studied. The funiculus is not vascularized but is the alternative route of translocation. A mechanical response to the collective size increase of the embryos is another possible cause.

As previously pointed out growth in diameter takes place in at least three well defined phases. Within two days after pollination a series of nuclear and cell divisions occurs within the inner wall layers of the ovary cavity. These divisions are mainly periclinal, few longitudinal. Except for another series of divisions occurring within the epidermal and hypodermal layers and scattered divisions in the regions adjoining the vascular bundles, all taking place several weeks later, there is no increase in the number of cells making up the fruit coat. The enlargement of cells already present must cause the major part of the increase in fruit wall thickness.

A study of changes in the fruit wall shows that this increase takes place early and can only partially explain the initial phase of growth in diameter. The increase in the diameter of the ovary cavity, however, has at least three growth phases exactly corresponding to the gross diameter changes. Since the growth of the cells of the inner layers of the ovary is periclinal, the substances in the pollinia and produced by the pollen tubes, or produced by the pollen tubes alone, probably regulate this growth during the first phase of increase in length and diameter respectively. (The dependence of diameter increase upon pollen tubes is borne out by zonal growth of the ovary, the swelling advancing as the strands of pollen tubes grow downward.)

During the first phase of diameter growth placental proliferation eventually leads to the initiation of ovule rudiments. While the rate of diameter growth is decelerating, increasing numbers of these rudiments are found.

At the same time the hairs which serve later as elaters develop from the innermost cell layers of the sterile lobes. Although there is no sharp break from one morphological stage in the reproductive cycle to the next, macrosporogenesis is taking place in the greatest number of ovules while the diameter growth is at or near a minimum rate. The close of the first phase of growth in diameter may rest with disturbed food relations or other changes occasioned by the heavier demands of the macrospore mother cells undergoing reduction division at that time. During the ensuing phase of growth in diameter the macrogametophytes develop; at the close of this development the ovules elongate slightly before fertilization. Fertilization takes place during a second period of minimum rate of growth in diameter. During the next few weeks the ovules increase in both dimensions while the embryos are growing; at this time the ovary undergoes the third phase of diameter increase. The pollen tubes disintegrate and disappear shortly after fertilization. It appears probable that the material derived from the pollen tubes functions either as a stimulus or as an actual energy source for the third increase in diameter of the ovary. The latter interpretation is supported by the known high content of pentosan-like hydrophilic colloids in the active microgametophyte.

It is noteworthy that each of the steps in internal development—(1) placental proliferation and ovule initiation, (2) maturation of the ovule in preparation for fertilization, and (3) seed development (embryo growth)—which demand more space are provided for by three cycles of diameter growth. At the time of macrosporogenesis and at the time of fertilization the rate of diameter growth is slow followed by an increased rate.

At the time of fertilization a group of sterility factors makes its appearance. If for genetical or cytological reasons macrosporogenesis fails, no macrogametes can be formed and fertilization cannot take place, even though the ovules elongate and there is a second phase of growth in diameter. A similar result would be obtained if the pollen tubes failed to reach the ovules (which is most common), if the act of fertilization itself failed, or if the zygotes or young embryos degenerated. (The failure of the *Phalaenopsis schilleriana* series at the Morris Arboretum was caused by some one of this group of factors.) No further growth occurs if fertilization does not take place; it follows that the third phase of diameter growth is a response to the fertilization stimulus. It seems that if fertilization took place and embryos developed to the two- or three-celled state before a degeneration set in that a third growth phase would follow. We have no data on this point.

Since the *Phalaenopsis* fruit approaches a cylinder in shape, computing the volume from length and diameter measurements is feasible. Any error involved, such as the slight tapering of the ovary base, would be more or less uniform. A growth curve erected (volume vs. time) very nearly approaches

—up to the time of embryo development—the logarithmic growth curve. A curve (fig. 4) representing increase in fresh weight follows the volume increase more closely throughout than it does curves representing either growth in length or diameter. The specific gravity decreases as the fruit matures, in consequence of the increased amount of air space within it, the decrease becoming most noticeable as the macrogametophytes mature.

The interpretation as presented is an elaboration and refinement of the ideas expressed by Crüger (1851) when he pointed out the "dynamic" effect of pollination in addition to the necessity of pollination prior to fertilization, and by Hildebrand (1863) when he demonstrated the "twofold" effect of pollination. While Brown (1831), Crüger, Hildebrand, and Guignard (1886) all commented on this dynamic effect of pollination, which is so obvious in the orchids since it precedes fertilization by a long period of time, and while the latter two knew that the ovary was not ready for fertilization at the time of pollination, they made no correlations between internal development and the various growth phases. Table 2, presenting the sequence of

TABLE 2

Summary of fruit changes and their possible causes

Cause	Result	Comment
1. Pollination effect.	1. Perianth wilting. 2. Increase in ovary curvature. 3. Initiation of nuclear and cell divisions in inner parietal layers of ovary. 4. Initiation of growth in both length and diameter.	The growth initiated is closely associated with following phases. Can only be dissociated in incompatible crosses. Its effect can be duplicated by the application of growth regulatory substances to the stigma.
2. Effect of entrance of pollen tubes into ovary.	1. Prevention of fruit drop.	Fruit set placed here because flowers in incompatible crosses, even though pollen germinates, fall off the inflorescence sooner than unpollinated flowers of same age.
	2. Grand phases of growth in both length and diameter.	At time of placental proliferation. Culminates in initiation of ovule rudiments with respect to diameter growth.
3. (Cause not clear)	1. A second phase of growth in diameter.	At time of macrogametophyte maturation. Cannot be dissociated experimentally from preceding growth phase in diameter. Cause may lie in ovary itself.
4. Fertilization effect.	1. A third phase of diameter growth and at times a second in length.	Not present if fertilization fails. A group of sterility factors separates this from preceding growth phases. Seed development at this time.

growth in relation to causality and to morphological changes, is self-explanatory.

Figure 9, a generalized curve of the increments of growth in diameter, shows a possible analysis into three component curves. Another component, the growth caused by the presence of pollen alone, which would occur at the beginning, is not separated from the first phase of growth because there is no method of judging from incompatible crosses the total extent of this effect. The general appearance of each segment is that of a normal distribution curve and may be so considered for the rate of the process concerned (ovule rudiment formation, macrogametophyte maturation, or embryo development) with the exception that the first is incorrect in time of initiation since the pollen effect is not segregated from it.

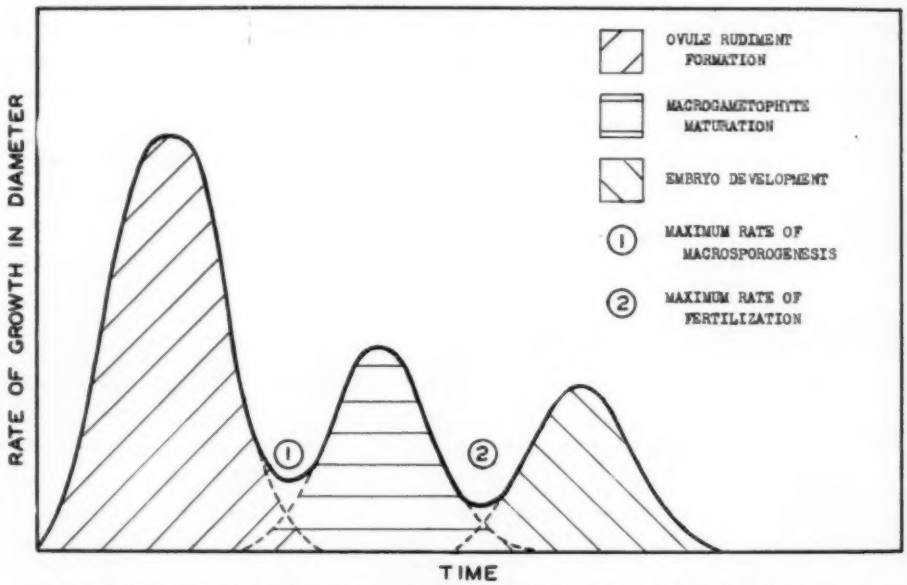


FIG. 9. Generalized curve based on weekly increments of growth in diameter of fruits of *Phalaenopsis*.

The results, now being prepared for publication, of similar investigations on numerous tropical orchids show highly comparable sequences of events. It is not thought, however, that all, or perhaps any, temperate orchids will do likewise. In such forms as *Orchis* (Hildebrand 1863) the ovary is farther advanced at time of pollination in that ovule rudiments are present. The recognition of similar growth cycles in the four genera of the *Cypripedilineae* may be more difficult in that ovule rudiments are present and in that the fairly large ovary cavity, present at time of pollination, allows space for considerable internal development without external manifestations.

Examination of data gathered by other workers on fruit development in forms not members of the *Orchidaceae* almost universally shows a break in the growth curve. Such breaks have been interpreted in three ways. First, they have been ignored and an exponential growth curve has been erected; second, they have been interpreted as an indication that at such a time individual parts of the ovary were growing at slightly different rates; third, they have been considered to indicate a fundamental physiological change. The present study goes beyond the last view in suggesting that there is a close correlation in the orchids between the growth rate as measured externally and the internal state of development.

Lilleland (1931) found a retarded period separating two periods of rapid growth in diameter of the pericarp of the apricot. This is considered characteristic of drupaceous fruits. Since pomes lack this retarded period, its cause has been ascribed to competition for food substances by the fleshy and stony layers of the pericarp. Tukey's (1933) graphs of the development of the cherry fruit show a deceleration of the rate of pericarp growth at the time endosperm and embryo are initiating their grand phase of development. However, it is highly improbable, because of the relative immaturity of the *Phalaenopsis* ovary at the time of pollination, that any close comparison can be drawn between fruit development in other Angiosperms in which it is well known and *Phalaenopsis*. Even in such genera as *Alnus* (Wolpert 1910), in which a period of three or four weeks elapses between pollination and fertilization, ovules, although not ready for fertilization, are present in some stage of development at the time of pollination. Furthermore the embyo sac reaches maturity even though the flower remains unpollinated. In general *Phalaenopsis* and other Angiosperms have pollination and fertilization effects in common, but the growth phases in *Phalaenopsis* lying between pollination and fertilization and having to do with placental proliferation, ovule initiation, and ovule maturation would probably come prior to the growth phase initiated by pollination in the ordinary Angiosperm. To state the assumption in another way: a pollination effect followed immediately by and perhaps indistinguishably united with a fertilization effect would be found in those Angiosperms which possess mature or nearly mature ovules at the time of pollination.

Strictly comparable studies on the growth phases of the ovaries of other Angiosperms are lacking. Likewise it remains to be seen how universal is this type of ovary development in the orchids. The appearance of mature ovules in *Phalaenopsis* long after pollination offers novel opportunities for experimental manipulations (including injection with various substances) at various critical points of its development as can be determined from the configuration of the growth curve.

SUMMARY

The ovary of *Phalaenopsis* has intermittent development.

The presence of the pollinia on the stigma brings about: (1) wilting of the perinath; (2) an initiation of column growth so that the pollinia are enclosed by the edges of the stigmatic cavity; (3) a change in the curvature of the ovary; (4) the initiation of nuclear and cell division in the cells in the inner layer of the ovary wall and in the cells of the placentae; and (5) the initiation of growth in both length and diameter.

Fruit set is determined by the entrance of the pollen tubes into the ovary cavity. At the same time added impetus is given to growth in length and diameter.

Ovary development is marked by three well defined phases of growth in diameter and two in length. The phases of growth in diameter coincide with morphological changes within the ovary, namely: (1) placental proliferation and ovule initiation, (2) preparation of the ovule for fertilization, and (3) embryo growth. The first of the phases of length growth closes at the time of fertilization; the second takes place at the time seeds are maturing. Critical points such as macrosporogenesis and fertilization take place while growth in diameter is near a minimum.

Incompatibility factors are expressed at the close of pollination effects. A type of sterility resulting in premature ripening of the fruit and following failure in macrosporogenesis or of the pollen tubes to reach the ovules was likewise found.

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VITAMIN DEFICIENCIES OF CERATOSTOMELLA

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It is now well established that the growth of many fungi is limited by their inability to make adequate quantities of one or more vitamins or vitamin-like growth substances. Besides being heterotrophic in relation to carbon these organisms exhibit various degrees of heterotrophy for growth substances. Such fungi do not grow or grow poorly in a solution of pure sugar, minerals, and asparagine, but they develop satisfactorily on the addition to this medium of various substances of natural origin, or of one or more chemically pure vitamins. Some of these fungi suffer from complete or major vitamin deficiencies,[1] that is, they make no growth unless the needed vitamins are supplied; others suffer from minor or partial deficiencies, that is, they grow slowly in the absence of the vitamin, but grow much more rapidly if some of the vitamin is present in the medium. Some fungi have a single (partial or complete) deficiency, but others may be afflicted with two or more.

The genus *Ceratostomella* is particularly interesting because it illustrates all of the generalities expressed above when the relation of some of its species to thiamine (vitamin B_1), pyridoxine (vitamin B_6), and biotin (vitamin H) is studied.

The genus *Ceratostomella* includes the causal organism of the Dutch elm disease, the cause of a serious disease of the London plane tree, and some fungi responsible for diseases of a variety of forest trees. Some species are known as the blue-stain fungi.

Through the courtesy of Dr. Carl Hartley and Dr. Caroline T. Rumbold we received cultures of the following species or strains of *Ceratostomella*.

C. ips Rumbold No. 255 associated with *Ips avulsus* in *Pinus echinata* and collected near Asheville, North Carolina (6, 7).

C. ips Rumbold No. 438 A associated with *Ips integer* in *Pinus ponderosa* and collected near Sisters, Oregon (6, 7).

C. pini Munch No. 512 associated with *Dendroctonus fontalis* in *Pinus echinata* and collected near Heleyville, Alabama (6, 7).

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[1] Two classes of vitamin deficiencies should be distinguished. One is physiological, an inability of the organism to synthesize a particular vitamin from simple foods and nutrients, for example, from sugar, minerals, and a source of nitrogen; the other is a vitamin deficiency in the food. Only those organisms which are physiologically deficient can suffer from a vitamin deficiency in the food. Schopfer has used the somewhat awkward term auxoheterotrophic to describe organisms physiologically deficient for one or more vitamins, and auxoautotrophic for those which are not deficient. It is the physiological deficiencies which chiefly concern us in this paper.

C. pini Munch No. 416 associated with *Dendroctonus breviconus* in *Pinus ponderosa* and collected near Sisters, Oregon (6, 7).

C. piceaperda Rumbold No. 240 associated with *Dendroctonus piceaperda* in *Picea glauca* and collected in Nova Scotia (7).

C. pseudotsugae Rumbold No. 436 associated with *Dendroctonus pseudotsugae* in Douglas fir and collected near Metaline Falls, Washington (7).

C. montium Rumbold No. 424 associated with *Dendroctonus monticolae* in *Pinus monticola* and collected near Metaline Falls, Washington (8).

Included in the study reported here were cultures of *C. ulmi* and of a *Ceratostomella* isolated from the London plane tree obtained through the kindness of Dr. James M. Walter, and a culture of *C. fimbriata* isolated from sweet potato and supplied by Dr. L. L. Harter.

METHODS AND MATERIALS

The organisms were grown in pure culture in 125 ml. Erlenmeyer flasks containing 25 ml. quantities of a basal solution, solution 1, composed per liter of 50 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, and 2 g. asparagine. To this solution the following trace elements in p.p.m. were added: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, and 0.09 Zn. The dextrose was Corn Products C.P. The asparagine was purified by treatment with Norit A and recrystallization from redistilled alcohol. All other chemicals were of chemically pure grade.

Merck's synthetic thiamine and pyridoxine were used; pure biotin methyl ester was obtained through the courtesy of Dr. Vincent du Vigneaud and a preparation from the SMA corporation was also used. Other vitamins or special chemicals employed are mentioned later in the text.

The fungi were grown also on agar slants in test tubes, each containing approximately 8 ml. of medium. The basal medium in these cultures consisted of solution 1 plus the mineral supplements solidified with 1.5 per cent Difco agar or 1.5 per cent agar which had been purified by extraction with aqueous pyridine and alcohol as previously described (5).

Pyrex glass thoroughly cleaned with a chromic–sulfuric acid mixture and washed with tap and distilled water was used throughout the experiments. The media were sterilized in an autoclave for 20 minutes at 15 lbs. pressure.

EXPERIMENTS

Response to Peptone. The Ceratostomellas grow readily on media supplemented with extracts of various natural products, for example, on malt agar, on agar containing an infusion of tree bark or on a thiamine-peptone agar (4). Preliminary investigation demonstrated that most of the 10 species or strains grew very poorly, or not at all, in a solution of dextrose, minerals,

asparagine, and thiamine, but grew quite well in the same medium containing peptone. This is illustrated in table 1. In the experiment summarized

TABLE 1. *Average dry weight in mg. of duplicate cultures of the Ceratostomellas indicated in a mineral-dextrose solution containing asparagine and thiamine, and in the same solution plus peptone*
Temperature 26° C., time of growth in each passage 15 days.

Additions to 25 ml. of solution 1	*C. piceaperda*	*C. ips*	*C. pini*	*C. montium*	*C. pseudotsugae*	*C. ips*	*C. pini*	*C. ulmi*
	No. 240	No. 255	No. 416	No. 424	No. 436	No. 438	No. 512	
10 μ g. thiamine first passage	1.8	0.2	11.3	0.2	57.9	2.4	1.6	0.1
10 μ g. thiamine and 25 mg. peptone 1st passage	44.5	27.3	52.7	14.4	76.4	39.4	38.2	16.9
10 μ g. thiamine 2nd passage	0.3	0.0	0.9	0.0	52.6	1.8	1.9	0.0
10 μ g. thiamine and 25 mg. peptone 2nd passage	45.6	25.4	52.5	13.3	66.9	39.4	38.4	18.5

there, duplicate cultures, each containing 25 ml. of solution 1 plus 10 μg. of thiamine or plus 10 μg. of thiamine and 25 mg. of neo-peptone, were inoculated from stock cultures grown on a thiamine-peptone agar. After 8 days growth at 26° C. sub-cultures were made into corresponding solutions for a second passage. Dry weights for both passages were determined after 15 days growth. *C. pseudotsugae* made considerable growth in the basal solution containing thiamine, but its growth was decidedly improved by the addition of peptone. The growth of the other 7 strains or species in the basal solution plus thiamine was limited to a milligram or two and was increased many times by the addition of peptone. It appeared, therefore, that peptone supplied these species of *Ceratostomella* with something necessary for growth which was not present in solution 1 plus thiamine.

Response to Thiamine, Pyridoxine, and Biotin. Further investigation in which 11 vitamins[2] and a number of amino acids were used, in various combinations, indicated that deficiencies of thiamine, pyridoxine, and biotin were primarily concerned in determining the growth of the Ceratostomellas studied.

The response of these fungi to the three vitamins was determined by growing each organism in 25 ml. of the basal solution and in the same medium plus biotin, thiamine, pyridoxine, biotin and thiamine, biotin and pyridoxine, pyridoxine and thiamine, or all three vitamins. The quantity of

[2] See footnote 3.

these vitamins used per flask was 0.1 μg. of biotin, 10 mμ moles of thiamine, and 50 mμ moles of pyridoxine. Each organism was grown also in the basal solution plus 0.3 g. of malt extract per flask. The experiment was performed in triplicate. The liquid cultures were inoculated from stock cultures on thiamine-peptone agar and incubated at 20° C. in the dark. Dry weights were determined after various periods of time, depending upon the rapidity of growth, as follows:

C. pseudotsugae No. 436, *C. ips* No. 438, after 8 days; *C. piceaperda* No. 240, *C. ips* No. 255, *C. pini* No. 416, *C. montium* No. 424, after 12 days; *C. pini* No. 512, after 14 days; *C. ulmi*, *C. fimbriata*, and the *Ceratostomella* from the London plane, after 20 days (table 2).

TABLE 2. *Average dry weight in mg. of triplicate cultures of the Ceratostomellas indicated in a basal mineral-dextrose solution containing asparagine to which thiamine, pyridoxine, and biotin were added alone or in combination*

Temperature of incubation 20° C. Time of growth ranged from 8 to 20 days; see text for details. First passage. Compare with table 3.

Additions to 25 ml. of solution 1	None	0.1 μg. biotin	50 mμ moles pyridoxine	10 mμ moles thiamine	pyridoxine and biotin	pyridoxine and thiamine	biotin and thiamine	pyridoxine, biotin, and thiamine	0.3 g. malt extract
C. pseudotsugae, No. 436	6.2	6.5	5.2	51.4	6.7	66.6	51.6	46.5	92.2
C. ips, No. 438	4.3	5.4	0.1	1.0	25.2	0.3	18.4	39.9	92.4
C. piceaperda, No. 240	0.2	2.8	3.3	0.2	8.2	17.6	16.9	27.5	48.4
C. ips, No. 255	no growth	no growth	no growth	no growth	1.9	0.1	0.1	52.6	51.5
C. pini, No. 416	0.2	2.2	0.1	0.1	1.2	0.1	35.6	34.9	32.0
C. montinum, No. 424	0.1	0.8	1.4	0.1	10.7	3.2	3.3	33.2	50.1
C. pini, No. 512	1.3	1.2	0.1	0.3	0.9	0.2	37.7	41.7	57.4
C. ulmi	no growth	no growth	25.1	no growth	24.0	25.2	no growth	25.2	119.7
C. fimbriata	2.1	4.5	0.1	32.4	0.6	60.6	59.9	103.6	91.6
C. from London plane	0.1	no growth	no growth	1.5	1.4	32.0	18.5	26.0	54.2

Sub-cultures from the growth in the first passage in liquid cultures were made into similar solutions for a second passage. This was in order to de-

crease the effect of whatever vitamins might have been present in the original inoculum which came from cultures grown on thiamine-peptone agar.

Each organism in the second passage (table 3) was grown also in the basal solution plus 11 vitamins and 21 amino acids[3] and in the basal solution plus 50 mμ moles of pyridoxine, 10 mμ moles of thiamine, and 19 mg. of neo-peptone per flask. For inoculating these cultures transfers from growth in the first passage in the basal solution was used for No. 436, No. 438, No. 424, No. 512, and *C. fimbriata*, from the basal solution supplemented with biotin for No. 240, No. 416, and *C. ulmi*, and from the basal solution supplemented with pyridoxine and thiamine for No. 255 and the *Ceratostomella* from the London plane.

Dry weights for the second passage (table 3) were determined after the same time intervals as in the first passage except for *C. ulmi*, *C. fimbriata*, and the *Ceratostomella* from the London plane, which were grown for 18 days only.

In addition the 10 organisms were grown in triplicate in test tubes on agar slants containing the basal solution solidified with 1.5 per cent purified agar and in the same medium plus per tube the same quantities and combinations of thiamine, pyridoxine, and biotin used in the liquid cultures. The cultures were incubated in the dark at 20° C. After 11 days sub-cultures were made to corresponding media. The results in the second passage on agar did not differ significantly from those observed in the first passage.

The results on the agar cultures and in the liquid media were as follows:

C. ips, No. 438, did not grow on the basal medium solidified with 1.5 per cent purified agar. The addition of thiamine or pyridoxine alone or together was of no benefit. The addition of biotin, biotin and thiamine, biotin and pyridoxine or of biotin, pyridoxine, and thiamine permitted good growth (fig. 1). It appeared from these results that *C. ips* No. 438 suffered from a complete or nearly complete biotin deficiency.

This conclusion was confirmed by experiments in liquid culture, which showed in addition that partial deficiencies for pyridoxine and thiamine probably exist for this organism. Very little growth occurred in the basal solution (tables 2, 3). The addition of biotin increased the growth several times. The quantity of pyridoxine and of thiamine used appeared to be somewhat injurious, for less dry matter was obtained in the cultures supple-

[3] The following amino acids were added in μg. per flask: d arginine 10, dl alanine 3, l aspartic acid 10, d glutamine 22, glycine 3, β alanine 80, l leucine 16, dl lyeine HCL 32, dl norleucine 16, serine 16, dl theonine 16, dl valine 16, d cysteine HCl 16, l histidine HCl 32, l hydroxyproline 16, dl methionine 16, dl phenylalanine 16, l proline 16, l tryptophane 32, tyrosine 8, thyroxine 8.

The following vitamins were added per flask: ascorbic acid 12 μg., pimelic acid 6 μg., para-amino-benzoic acid 6 μg., vitamin K_1 6 μg., calcium pantothenate 6 μg., lactoflavin 10 mμ moles, nicotinamide 10 mμ moles, m-inositol 1 mg., pyridoxine 50 mμ moles, thiamine 20 mμ moles, biotin 0.1 μg.

mented with thiamine or pyridoxine than in the basal solution. Pyridoxine and biotin together or thiamine and biotin together were 3 to 5 times as effective as biotin alone while the best growth was obtained with all three vitamins present in the medium. Growth in the media containing biotin was distinctly better in the second passage than in the first.

C. ulmi grew on the agar medium satisfactorily only in those tubes which contained pyridoxine. On the basal medium or on the basal medium containing thiamine or biotin, or a combination of the two, the development was slight (fig. 1). *C. ulmi* apparently has a complete pyridoxine deficiency.

These observations were supported by the results in liquid culture

TABLE 3. *Average dry weight in mg. of triplicate cultures of the Ceratostomellas indicated in a basal mineral-dextrose solution containing asparagine to which thiamine, pyridoxine, and biotin were added alone or in combination*

Temperature of incubation 20° C. Time of growth ranged from 8 to 18 days; see text for details. Second passage. Compare with table 2. See footnote No. 3 for the 11 vitamins and 21 amino acids used.

Additions to 25 ml. of solution 1	None	0.1 μg. biotin	50 mμ moles pyridoxine	10 mμ moles thiamine	pyridoxine and biotin	pyridoxine and thiamine	biotin and thiamine	biotin, pyridoxine and thiamine	Eleven vitamins and 21 amino acids	pyridoxine, thiamine, 19 mg. neopeptone
C. pseudotsugae, No. 436	5.1	4.5	10.3	69.9	12.6	64.5	78.5	73.7	82.4	85.4
C. ips, No. 438	2.7	16.9	1.0	0.8	65.0	2.2	68.6	77.8	76.2	31.9
C. piceaperda, No. 240	0.7	4.4	13.5	0.5	16.0	26.9	18.3	43.8	59.8	61.0
C. ips, No. 255	no growth	no growth	no growth	no growth	1.3	no growth	no growth	55.8	49.2	44.4
C. pini, No. 416	0.3	1.2	no growth	no growth	1.0	no growth	57.7	60.9	57.6	46.4
C. montinum, No. 424	0.3	3.5	0.6	0.5	3.4	2.3	0.2	48.1	55.1	44.1
C. pini, No. 512	0.1	0.6	0.4	0.4	0.8	0.1	34.2	39.2	64.8	40.8
C. ulmi	no growth	no growth	36.8	no growth	30.7	28.8	no growth	29.4	42.3	56.9
C. fimbriata	0.7	0.7	0.5	95.9	0.7	131.4	130.8	127.8	62.7	65.6
C. from London plane	0.3	0.1	0.2	14.6	0.1	34.9	37.9	40.2	44.2	68.2

(tables 2, 3). *C. ulmi* did not grow in the basal medium and the addition of thiamine or of biotin, alone or together, had no effect. The growth with

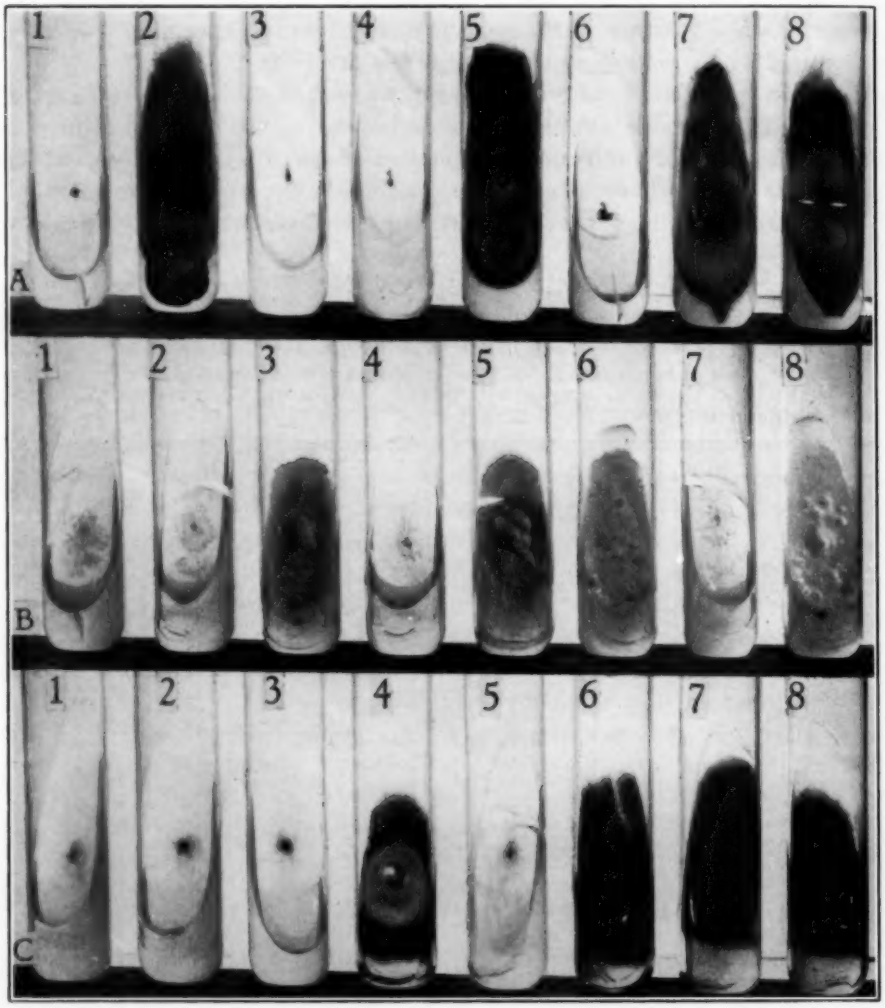


FIG. 1. Growth of various species of *Ceratostomella* on a basal mineral—dextrose medium containing asparagine and 1.5 per cent purified agar supplemented as follows: (1) no addition; (2) biotin; (3) pyridoxine; (4) thiamine; (5) biotin and pyridoxine; (6) pyridoxine and thiamine; (7) thiamine and biotin; (8) pyridoxine, thiamine, and biotin. A, *C. ips* No. 438; B, *C. ulmi*; C, *C. fimbriata*. Age of cultures 26 days.

pyridoxine, pyridoxine and thiamine, pyridoxine and biotin, and with all three vitamins was approximately the same, indicating under these conditions no partial deficiencies for biotin or thiamine.

Attempts to substitute nicotinamide, calcium pantothenate, m-inositol, or para-amino benzoic acid for pyridoxine were unsuccessful.

C. fimbriata grew very little in the basal agar medium and the addition of pyridoxine or of biotin alone and together had little effect. When thiamine was added growth was quite satisfactory (fig. 1); more rapid development occurred in those media supplemented with thiamine and pyridoxine, thiamine and biotin, or with the three vitamins. *C. fimbriata* appeared to have a complete deficiency for thiamine, and partial deficiencies for pyridoxine and biotin.

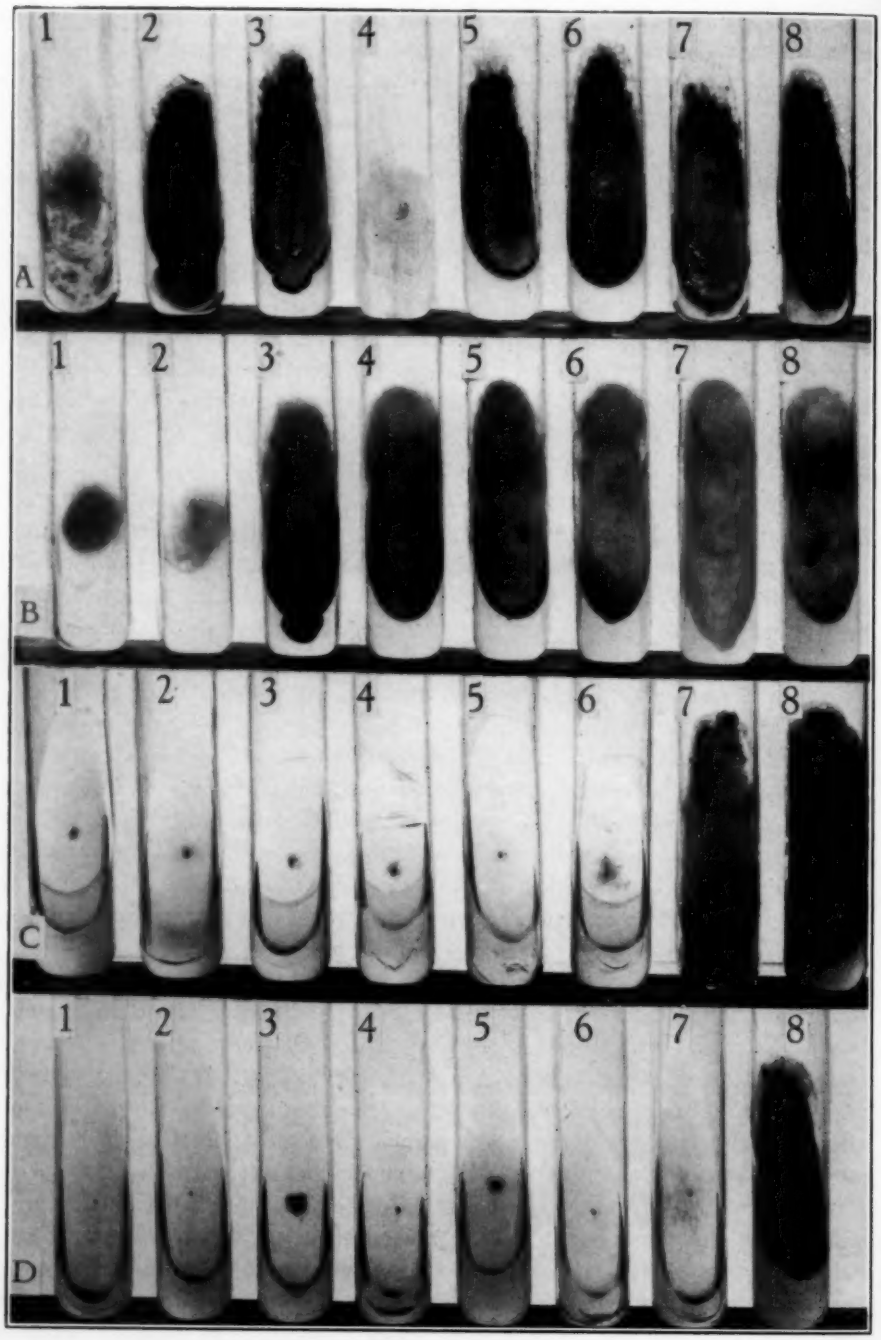
These conclusions were confirmed by the liquid cultures (tables 2, 3). Little growth was obtained in the basal medium or in the basal medium plus biotin or pyridoxine singly or together. Good growth was obtained on the addition of thiamine and was increased when thiamine and pyridoxine or thiamine and biotin were used as supplements. The best growth was obtained when all three vitamins were present.

The *Ceratostomella* from the London plane tree resembled *C. fimbriata* in its vitamin deficiencies. It grew less rapidly and may suffer from more marked pyridoxine and biotin deficiencies than *C. fimbriata* (tables 2, 3).

C. piceaperda, No. 240, grew slowly on the basal agar medium. Little growth was visible after 5 days. After 7 days colonies 2–5 mm. in diameter had developed and after 10 days the colonies averaged 12 mm. in diameter. The addition of thiamine to the basal medium had some, though little, beneficial effect on the early growth, but the addition of biotin or pyridoxine materially increased the rapidity of growth (fig. 2). The tubes containing biotin or pyridoxine after 5 days had colonies from 33 to 50 mm. in diameter and after 10 days the agar slopes were entirely covered with mycelium. The most rapid growth occurred in the tubes containing all three vitamins. The cultures on agar indicated that *C. piceaperda* suffers from partial deficiencies for biotin and pyridoxine. When these deficiencies were satisfied the addition of thiamine had a further beneficial effect.

C. piceaperda grew very little in the basal solution and the addition of thiamine had little effect (tables 2, 3). Biotin increased growth somewhat but pyridoxine had a much greater effect. Pyridoxine and biotin together were about as effective as pyridoxine alone and about equal to the mixture of biotin and thiamine. Pyridoxine and thiamine together were still more effective and the three vitamins combined were most effective. These comments are based on the results of the second passage (table 3).

C. piceaperda grew on the basal agar medium and acted as though it suffered chiefly from partial biotin and pyridoxine deficiencies. In liquid culture it grew little or not at all in the basal medium and appeared to have a complete deficiency for biotin and pyridoxine and a partial thiamine deficiency. The apparent differences between the growth of *C. piceaperda*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on agar and in liquid culture may be the result of more rapid development on agar. It was noted that the relative growth in the second passage in the liquid cultures at the end of 12 days was much the same as that observed on agar at the end of 6 days.

C. pseudotsugae, No. 436, grew slowly on the basal agar medium. At the end of 5 days this fungus had developed colonies 19 mm. in diameter and after 12 days the colonies were 39 mm. in diameter. The addition of biotin did not increase the rapidity of growth but pyridoxine had some beneficial effect and thiamine was distinctly beneficial. The combination of biotin and pyridoxine was more beneficial than pyridoxine alone. Good growth was obtained also on the media containing pyridoxine and thiamine, biotin and thiamine, or all three vitamins (fig. 2). *C. pseudotsugae* seemed to have partial deficiencies for thiamine and pyridoxine.

In the first passage in liquid culture there was no evidence for a pyridoxine deficiency, but in the second passage partial deficiencies for thiamine and pyridoxine were indicated by the increased growth obtained when these vitamins were supplied (table 3).

C. pini, No. 512, did not grow on the basal agar medium and the addition of biotin, of pyridoxine, of thiamine, of biotin and pyridoxine together, or of pyridoxine and thiamine together had little or no effect (fig. 2). The addition of biotin and thiamine or of all three vitamins permitted good growth. From these results it appeared that this fungus suffers from complete biotin and thiamine deficiencies.

The liquid cultures confirmed this assumption. Little growth was obtained in any of the solutions except those supplemented with both biotin and thiamine or all three vitamins. The somewhat greater growth obtained when all three vitamins were present suggests that there may be also a partial pyridoxine deficiency (table 3).

C. pini, No. 416, responded much as did the isolation of the same species from *Pinus echinata*. It grew somewhat more rapidly and differed somewhat in gross morphology (tables 2, 3).

C. ips, No. 255, did not grow on the basal agar medium. The addition of biotin produced a small amount of growth but thiamine or pyridoxine was entirely ineffective. When the vitamins were added to the basal medium in pairs thiamine and pyridoxine were entirely ineffective and the growth with biotin and thiamine or with biotin and pyridoxine was about the same as

Explanation of figure 2

FIG. 2. Growth of various species of *Ceratostomella* on a basal mineral—dextrose medium containing asparagine and 1.5 per cent purified agar supplemented as follows: (1) no addition; (2) biotin; (3) pyridoxine; (4) thiamine; (5) biotin and pyridoxine; (6) pyridoxine and thiamine; (7) thiamine and biotin; (8) pyridoxine, thiamine, and biotin. A, *C. piceaperda*, No. 240; B, *C. pseudotsugae*, No. 436; C, *C. pini* No. 512; D, *C. ips* No. 255. Age of *C. pseudotsugae* 12 days; others, 26 days.

with biotin alone. When all three vitamins were added the growth was quite satisfactory. *C. ips,* No. 255, appeared to have complete or nearly complete deficiencies for biotin, thiamine, and pyridoxine, and in order to obtain good growth all three vitamins had to be added to the medium (fig. 2).

These results were essentially duplicated in the liquid cultures (tables 2, 3). In the second passage 55.8 mg. of mycelium were produced in the presence of all three vitamins while the maximum obtained with any single one or combination of two was 1.3 mg.

It is interesting to contrast this strain of *C. ips* with No. 438 which showed a single major deficiency—one for biotin.

C. montium, No. 424, closely resembled *C. ips,* No. 255, in its vitamin needs on the agar media. It appeared to have complete or nearly complete deficiencies for all three vitamins. This was true also for the liquid cultures (tables 2, 3). Little growth occurred in the basal solution, biotin improved the situation a little, but the growth was insignificant until all three vitamins were added.

The results given above indicate that these 10 fungi, all belonging to the genus *Ceratostomella,* have 7 distinct combinations of vitamin deficiencies under the conditions of our experiments. These may be tabulated as follows:

TABLE 4. *Vitamin deficiencies of 10 species or strains of Ceratostomella*
Note that the 10 fungi show 7 different types of deficiencies for the 3 vitamins. For *C. piceaperda* the partial deficiencies for biotin and pyridoxine are the more marked.

Fungus	Deficiency for		
	Biotin	Pyridoxine	Thiamine
C. ips No. 438	complete	partial	partial
C. ulmi	none	complete	none
C. fimbriata	partial	partial	complete
C. from London plane	partial	partial	complete
C. piceaperda No. 240	partial	partial	partial
C. pseudotsugae No. 436	none	partial	partial
C. pini No. 512	complete	partial	complete
C. pini No. 416	complete	partial?	complete
C. ips No. 255	complete	complete	complete
C. montium No. 424	complete	nearly complete	nearly complete

Synthesis of Vitamins by Ceratostomella. All the available evidence indicates that each living organism requires many vitamins. An organism which shows a deficiency for one is capable of synthesizing the others from the more elementary foods and nutrients, for example, from sugar, minerals, and a nitrogen source. It seemed worth while to test this assumption for the organisms and the vitamins concerned in this study.

C. fimbriata grew in a medium supplemented with thiamine. Is it capable of making biotin and pyridoxine from sugar, minerals, and asparagine (and thiamine)? The vitamin deficiency for *C. ulmi* is pyridoxine. Can it synthesize thiamine and biotin?

The procedure in answering these questions for *C. fimbriata* was as follows:

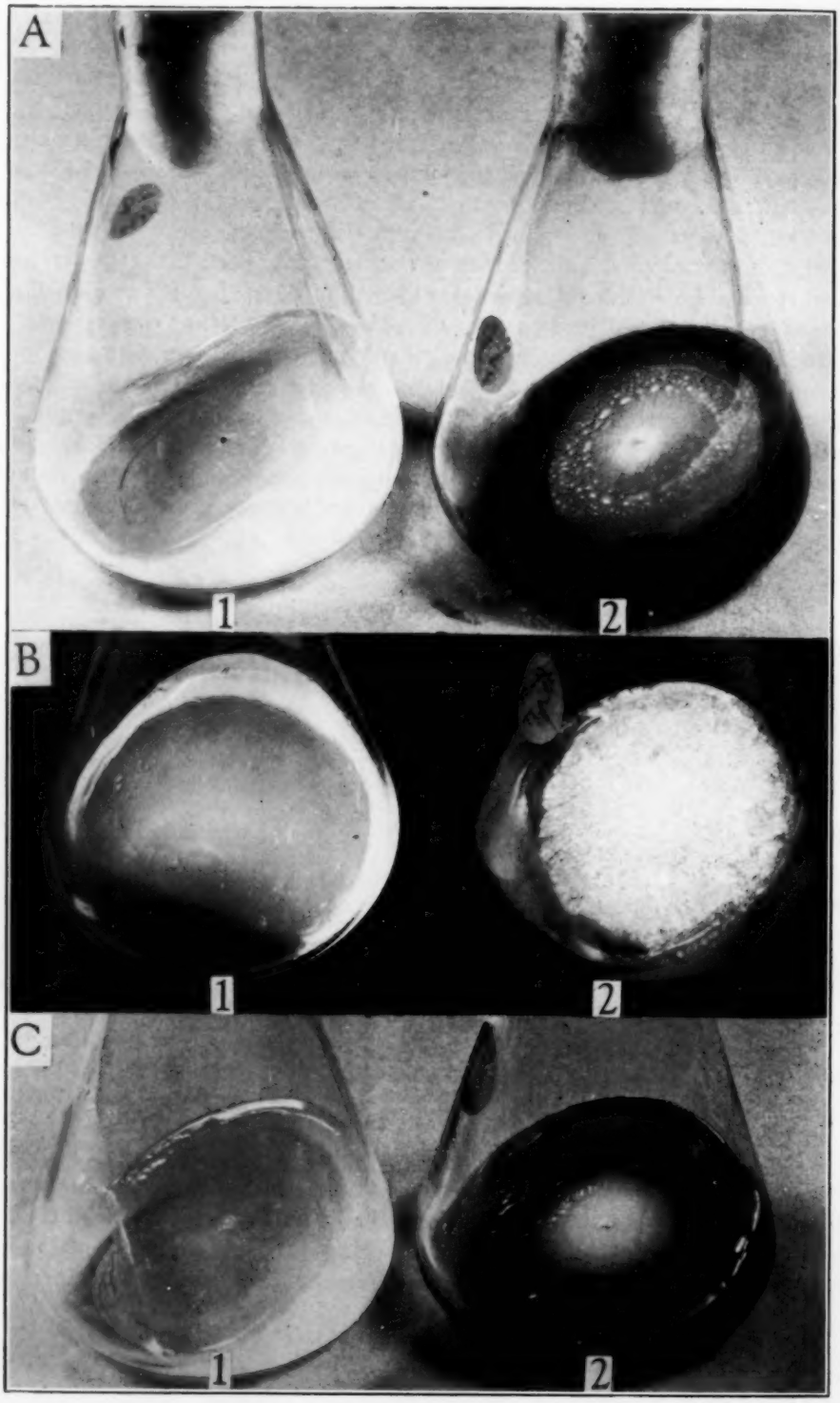
C. fimbriata was grown in 25 ml. quantities of the basal solution plus 10 mμ moles of thiamine per flask. At the end of 9 days and without the removal of the mycelium sufficient purified agar was added to each flask to make a 1.5 per cent solution and the solution was sterilized. The agar medium in some flasks was inoculated with *C. ulmi*, in others with *C. ips* No. 438, and in others with *C. montium* No. 424. Since the original medium contained thiamine but no pyridoxine, growth of *C. ulmi* would show that *C. fimbriata* had synthesized pyridoxine. Similarly growth of *C. ips* No. 438 would show that *C. fimbriata* had made biotin. *C. montium* No. 424 has complete deficiencies for all three vitamins and its growth on a medium to which thiamine only had been originally added would demonstrate that *C. fimbriata* had synthesized both pyridoxine and biotin. The results showed clearly the synthesis of pyridoxine and biotin by *C. fimbriata* from sugar, minerals, and asparagine (and thiamine) (fig. 3). By an analogous procedure the synthesis of vitamins by other species of *Ceratostomella* was determined as shown in the following table. All tests were positive.

TABLE 5. *Various species or strains of Ceratostomella tested for synthesis of vitamins*

The vitamins tested for and the organisms used in the bio-assay are given. The results of all tests were positive.

Fungus and culture solution tested	Tested for	Tested with
C. fimbriata grown in soln. 1 plus thiamine	pyridoxine biotin pyridoxine and biotin	*C. ulmi* *C. ips* No. 438 *C. montium* No. 424
Ceratostomella from London plane grown in soln. 1 plus thiamine	pyridoxine biotin pyridoxine and biotin	*C. ulmi* *C. ips* No. 438 *C. montium* No. 424
C. ulmi grown in soln. 1 plus pyridoxine	biotin thiamine biotin and thiamine	*C. ips* No. 438 *C. fimbriata* *C. montium* No. 424
C. ips No. 438 grown in soln. 1 plus biotin	pyridoxine thiamine	*C. ulmi* *C. fimbriata*
C. pini No. 512 grown in soln. 1 plus biotin and thiamine	pyridoxine pyridoxine	*C. ulmi* *C. montium* No. 424
C. pini No. 416 grown in soln. 1 plus biotin and thiamine	pyridoxine pyridoxine	*C. ulmi* *C. montium* No. 424

Other Vitamin Deficiencies. It is possible to grow any of the 10 fungi studied in a basal medium supplemented with thiamine, pyridoxine and biotin. Do these three vitamins satisfy all the vitamin deficiencies of all these organisms? It is probable that they do not. It is probable that some of these organisms suffer from partial deficiencies for other unidentified vitamins.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This is suggested by the development of perithecia on the basal agar medium supplemented with the three vitamins as compared with that described by Rumbold on malt agar. Several of the fungi did not form perithecia on our synthetic media and none developed these bodies so rapidly as has been described for their formation media containing natural supplements. Of course, we cannot on the basis of our present knowledge ascribe the slowness or the failure of perithecial development to deficiencies for vitamins other than thiamine, biotin, and pyridoxine. A number of other factors may be concerned. However, the perithecial development indicates that the basal medium supplemented with the three vitamins is probably not optimal for all these organisms and partial deficiencies for one or more additional vitamins may be concerned.

Further evidence for partial deficiencies of additional vitamins is found in the relative growth of some of these fungi in the basal medium supplemented with the three vitamins and in media containing additional vitamins or natural products.

For example, *C. ulmi* produced a dry weight of 25.2 mg. in the basal medium plus thiamine, pyridoxine, and biotin, but with malt extract the yield was 119.7 mg. (table 2). In the basal medium containing thiamine, pyridoxine, and peptone, the yield was 56.9 mg., as compared to 29.4 mg. in the basal medium containing thiamine, biotin, and pyridoxine (table 3). One explanation for the benefit noted from malt extract and from peptone on the growth of *C. ulmi*, which should be investigated, is that these natural products supply additional vitamins.

The growth of *C. piceaperda* No. 240 was improved by the addition of calcium pantothenate, nicotinamide, and para-amino benzoic acid to a basal solution containing biotin, thiamine, pyridoxine, and m-inositol. Still greater improvement was observed with the further addition of lactoflavin, ascorbic acid, pimelic acid, and glutamine. This suggests that partial deficiencies for other vitamins exist for this organism. In fact, greater growth was obtained in a medium supplemented with additional vitamins or with some natural product for all the *Ceratostomellas* except *C. ips* No. 255, *C. pini* No. 416, and *C. fimbriata*.

The addition to the basal medium of 11 vitamins and 21 amino acids did not improve the growth of No. 438, No. 255, No. 416, *C. fimbriata*, and the *Ceratostomella* from the London plane (table 3), as compared to that in the basal medium plus biotin, pyridoxine, and thiamine. However, the basal

Explanation of figure 3

FIG. 3. Production of biotin and pyridoxine by *C. fimbriata*. (1) basal medium containing thiamine solidified with purified agar; (2) same medium in which *C. fimbriata* was grown also solidified with purified agar. A, inoculated with *C. ips* No. 438; B, inoculated with *C. ulmi*; C, inoculated with *C. montium* No. 424.

medium plus malt extract was more effective than the basal medium plus thiamine, biotin, and pyridoxine for No. 438 and the *Ceratostomella* from the London plane. Furthermore, the beneficial effect of the malt extract of *C. ulmi* was much greater than that of the 11 vitamins and 21 amino acids. This suggests that *C. ips* No. 438, the *Ceratostomella* from the London plane, and *C. ulmi* suffer from deficiencies not supplied by the 11 vitamins and 21 amino acids.

The possibility that partial deficiencies for other vitamins than biotin, thiamine, and pyridoxine exist for some of these organisms and the conditions for good perithecial development should be investigated further.

Effect of Vitamins on Gross Morphology. The nature of the vitamins supplied influenced the pigment production and the character of the growth in several instances. For example, *C. fimbriata* developed on agar a white cottony mycelium when supplied with thiamine only, but with thiamine and biotin, with thiamine and pyridoxine, or with all three vitamins the mycelium was moist with a white frosting. In liquid culture after 8 days the same organism in the solution supplemented with thiamine showed a single submersed mycelial colony about 1 cm. in diameter, but with thiamine and pyridoxine, thiamine and biotin, or all three vitamins the surface of the liquid was covered with a dry torula-like growth in addition to the submersed mycelium.

C. ips No. 438 formed a dry mycelium on agar supplemented with biotin, while on agar supplemented with biotin and thiamine, biotin and pyridoxine, or with all three vitamins the growth was moist. The casual observer might easily have concluded that two different organisms were concerned.

Time did not permit a detailed investigation of these differences, some of them doubtless associated with the relative abundance of conidia. It is worth recording, however, that the supply of vitamins did markedly influence the gross morphology of some of these fungi.

Presence of Biotin, Pyridoxine and Thiamine in Cotton Batting and in Agar. In the course of an experiment not reported in detail here it was noted that *C. ips* No. 255 grew vigorously in one flask of three, which contained the basal solution plus thiamine and calcium pantothenate. Since this fungus suffers from complete thiamine, biotin, and pyridoxine deficiencies, it was evident that the last two vitamins must have been fortuitously introduced into this flask. Investigation showed that water had dripped during sterilization from the roof of the autoclave on the cotton stopper of this flask and percolated into the culture medium. Cotton batting was therefore investigated for biotin, pyridoxine, and thiamine.

A quantity of cotton batting was moistened with distilled water and autoclaved. The liquid was pressed out and made up so that 1 ml. of the

extract was equivalent to 1 g. of the cotton batting. No attempt was made to make a thorough and complete extraction of the cotton. The extract contained 12.2 mg. dry matter per ml.

To determine the presence of vitamins in this extract, tubes of the basal medium plus 1.5 per cent purified agar were prepared. To some tubes 1 ml. of the cotton extract was added. Tubes of the basal medium and of the basal

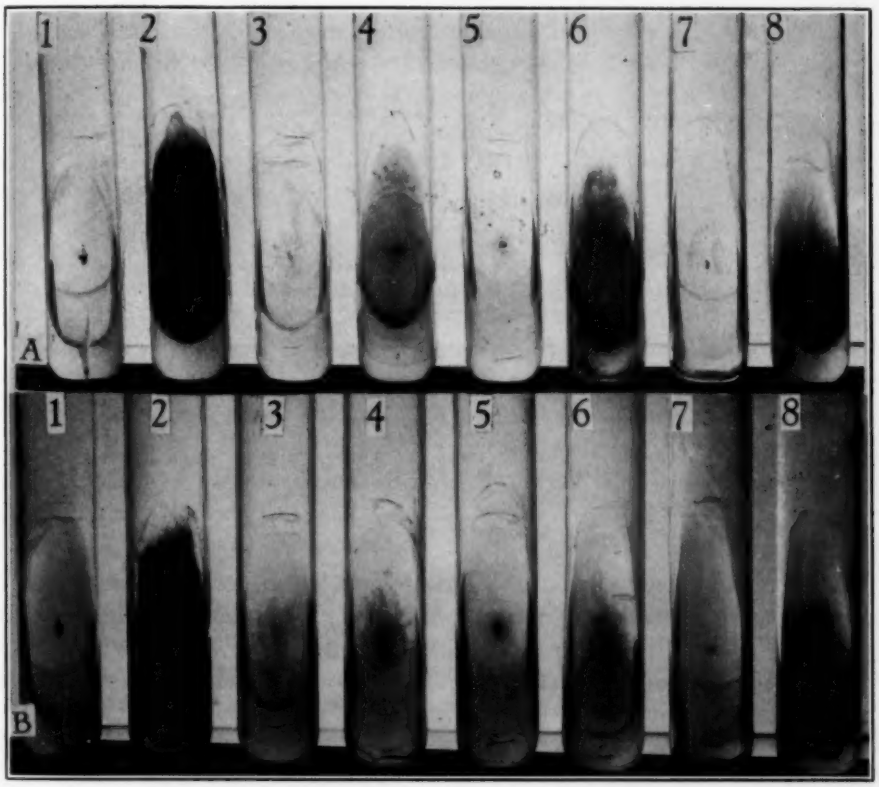

FIG. 4. Presence of biotin, pyridoxine, and thiamine in cotton extract and unpurified agar. A(1) basal medium with purified agar inoculated with *C. ips* No. 4338; A(2) same with extract from 1 g. cotton batting; A(3) basal medium inoculated with *C. ulmi;* A(4) same with cotton extract; A(5) basal medium inoculated with *C. fimbriata;* A(6) same with cotton extract; A(7) basal medium inoculated with *C. ips* No. 255; A(8) same with cotton extract. B(1–8) purified agar compared with unpurified agar.

medium plus the cotton extract were inoculated in triplicate with *C. ips* No. 438 to test for biotin, with *C. ulmi* to test for pyridoxine, with *C. fimbriata* to test for thiamine, and with *C. ips* No. 255 which grows well only when all three vitamins are present. The results (fig. 4) demonstrated that the cotton extract contained per ml. sufficient biotin, pyridoxine, and thiamine to give maximum or nearly maximum effects with these four species of *Ceratosmella.*

The cotton extract was used also in solution cultures. Growth of *C. ips* No. 438, of *C. ulmi*, of *C. montium* No. 424, and of *C. ips* No. 255 in the basal medium plus the cotton extract demonstrated the presence of thiamine, pyridoxine, and biotin in this material (table 6). The growth of *C. ips* No. 438 in the basal solution plus the extract of 3 g. of cotton was superior to that obtained in any other medium, including malt extract.

TABLE 6. *The effect of an extract of cotton batting on the growth of the Ceratostomellas indicated*

Grown 14 days at 20° C. The average dry weight of triplicate cultures is given in mg.

Additions to 25 ml. of soln. 1	*C. ips*	*C. ulmi*	*C. montium*	*C. ips*	*C. piceaperda*
	No. 438		No. 424	No. 255	No. 240
10 mμ moles thiamine	0.0	0.0	0.0	0.0	0.1
10 mμ moles thiamine plus extract 0.3 g. cotton	62.0	5.7	3.3	2.8	43.9
10 mμ moles thiamine plus extract 1.5 g. cotton	93.6	9.3	8.3	10.2	74.7
Extract 1.5 g. cotton	121.9	8.3	9.2	7.7	20.6
Extract 3.0 g. cotton	135.1	8.9	13.9	11.7	27.2
10 mμ moles thiamine, 0.01 μg. biotin, 50 mμ moles pyridoxine	10.6	9.2	17.6	11.6	50.5

We cannot be certain that these vitamins were present in the cotton boll and were not introduced in some way after the cotton was picked. The probability is that the former assumption is correct. In any event it is clear that cotton batting, so freely used as a laboratory material, may supply various vitamins, including biotin, pyridoxine, and thiamine.

Difco agar also contains all three vitamins. This is demonstrated in figure 4. The four organisms used in the experiment on cotton extract were grown on the basal medium solidified on the one hand with 1.5 per cent purified agar and on the other with 1.5 per cent unpurified Difco agar. Each tube contained about 0.12 g. of dry agar. Although the greater growth of *C. ulmi* and of *C. fimbriata* on the unpurified agar demonstrated the presence of pyridoxine and thiamine, there was not sufficient of these vitamins in the amount of agar used to give a maximum effect. *C. ips* No. 438 grew quite well on the unpurified agar, showing the presence of sufficient biotin to produce nearly a maximum effect on this fungus.

DISCUSSION

Earlier work on the nutrition of *Ceratostomella* furnishes little information on the nutritional requirements of the genus. Hedgcock (3) cultivated

a considerable number of species of *Ceratostomella* and found a pine decoction agar made from bark and sap wood to be an excellent medium, considerably superior to an agar made from heart wood. Schwarz (9) states that *C. ulmi* did not grow on a nutrient base which contains sugar as the only source of carbon. He grew this organism also on media containing a variety of natural products. It is clear now that *C. ulmi* failed to grow in some of the media used by Schwarz because the medium lacked pyridoxine and not because sugar was the only source of carbon. Clinton and McCormick (2) grew *C. ulmi* on a variety of natural media including elm twigs, cherry agar, plugs of potato and carrot, grains of wheat, oats and barley, sterilized liver, elm bark, and elm wood. Evidently all of these materials contained sufficient pyridoxine to permit growth. Rumbold (6, 7, 8) used chiefly a malt extract agar in the cultivation of the species of *Ceratostomella* she studied. She (7) found *C. pini* grew abnormally on a mineral—dextrose medium containing 1.5 per cent agar and either sodium caseinate or peptone and did not grow on the same medium containing asparagine or urea. It seems probable that lack of sufficient thiamine was partly responsible for these results. Rumbold (7) reported that *C. pseudotsugae* grew well on a mineral—dextrose medium containing 1.5 per cent agar and urea, asparagine, sodium caseinate, or peptone. We found that this organism grew on an agar medium to which no vitamins were added, especially if Difco agar rather than purified agar was used. Taylor-Vinje (10) grew *C. montium* on a corn meal decoction solidified with agar. Bramble and Holst (1) grew *C. pini* on a malt agar or malt broth. Our results show that the advantage of media containing natural products for the cultivation of *Cerastomella* is probably due to the vitamins supplied by the natural supplements.

Our results are of interest also because of the diversity of vitamin deficiencies evidenced by representatives of a single genus. It seems probable that a study of other members of this genus would add to this diversity. We might, for example, find a species which suffers from complete deficiencies for more vitamins than biotin, pyridoxine, and thiamine; one which would not grow even if all three of these vitamins were supplied.

Attention should be called to the vitamin relations of different isolations of the same species. The two isolations of *C. pini*, No. 416 and No. 512, were much alike in their vitamin deficiencies but the two isolations of *C. ips* No. 438 and No. 255, were decidedly different. The former (No. 438) was marked by a complete biotin deficiency and partial deficiencies for pyridoxine and thiamine, while the latter (No. 255) showed complete deficiencies for all three vitamins. Evidently these differences in vitamin deficiencies did not affect the characters differentiating the species.

Our observations are of interest also because of the deficiencies for pyridoxine shown by these organisms. Reports of pyridoxine deficiencies for

filamentous fungi have not hitherto appeared, yet three of the ten *Ceratostomellas* we studied showed a complete pyridoxine deficiency and seven a partial pyridoxine deficiency. *C. ulmi* is of special interest in this connection because it suffers from a pyridoxine (complete) deficiency only. This suggests that it might be used as a bio-assay for this vitamin. However, the character of its growth makes dry weight determinations tedious, and photoelectric determinations of the growth of this organism were not entirely satisfactory. Furthermore, it appears to have unidentified partial deficiencies which markedly affected its growth. What influence this might have on the use of *C. ulmi* for the quantitative determination of pyridoxine in natural products would need further study.

These experiments emphasize also the importance of partial vitamin deficiencies in limiting growth. A complete deficiency causes a marked and dramatic effect. If the vitamin is absent from the medium no growth occurs; its addition, if a single complete deficiency is concerned, permits growth. It is an all or none effect. On the other hand, partial deficiencies permit slow growth to take place in the absence from the medium of the vitamin concerned and its addition merely increases the rate of growth. Partial deficiencies are less obvious in their effects than complete deficiencies. Nevertheless, they both influence the rate and the character of the development of an organism.

Because of the possible influence of partial vitamin deficiencies the occurrence of growth on any given medium may not be taken as a priori evidence for eliminating vitamins as limiting factors. Seven of the ten species or strains studied in the experiments described in this paper were found to suffer from partial deficiencies of thiamine, pyridoxine, or biotin. Furthermore, some evidence for partial deficiencies of unidentified vitamins was found for two of the three remaining organisms. For this genus partial deficiencies are of general occurrence and considerable importance.

The interrelation between the various vitamins presents an interesting situation. *C. fimbriata,* for example, grows very little in the basal medium. The addition of thiamine (table 3) results in a great increase in growth and the synthesis of both biotin and pyridoxine (table 5). However, maximum growth is not obtained in the thiamine medium apparently because the organism does not synthesize enough biotin and pyridoxine, since the addition of biotin or pyridoxine or both to the thiamine medium results in greater growth. The situation is somewhat puzzling. If the biosynthesis of either biotin or pyridoxine limits growth in the thiamine medium then we should expect the addition of one to increase the growth and the other to be ineffective. If the synthesis of both biotin and pyridoxine is the limiting factor in the thiamine medium then it should be necessary to add both before any marked effect is obtained. However, both the combination of thiamine and pyridoxine and of thiamine and biotin are more effective than thiamine

alone. The accumulated evidence from other sources on the specificity of the vitamins would not encourage us to believe that pyridoxine takes the place of biotin or vice versa. We might assume that an increase in the supply of biotin in the presence of thiamine makes the synthesis of more pyridoxine possible and, on the other hand, an increased supply of pyridoxine permits a greater production of biotin. It is possible also that one vitamin is utilized more effectively when others are present in adequate amounts. A similar situation exists in the relation of thiamine and pyridoxine to the growth of *C. ips* No. 438 (table 3) in a medium containing biotin.

SUMMARY

Ten species or strains of *Ceratostomella* were grown in a basal mineral-dextrose medium containing asparagine and in the same medium supplemented with biotin, pyridoxine, or thiamine singly and in combination. All the fungi suffered from complete or partial vitamin deficiencies and 7 different combinations of deficiencies were found. The use of media containing natural products for the cultivation of these fungi is due to their vitamin deficiencies. Some evidence is presented for the existence of deficiencies for unidentified vitamins. The presence of biotin, thiamine and pyridoxine in cotton batting and in Difco agar was demonstrated.

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EFFECT OF VARIATIONS IN LIGHT INTENSITY, LENGTH OF PHOTO-PERIOD, AND AVAILABILITY OF NITROGEN UPON ACCUMULATION OF ASCORBIC ACID IN COWPEA PLANTS[1]

MARY ELIZABETH REID

An understanding of the causes of variations in the ascorbic acid content of plants is desirable from both practical and theoretical considerations. Of the various environmental factors known to influence accumulation of this vitamin in plants, light appears to have the greatest effect. Differences in intensity, quality, and length of the daily period of illumination may all be concerned in producing modifying effects.

The early investigations on the effects of light upon ascorbic acid accumulation were reviewed in a previous publication (Reid 1938). Murphy (1939) has shown that the ascorbic acid content of apples is higher on the side exposed to the sun than on the shaded side. Harding, Winston, and Fisher (1939) observed significantly higher values in oranges picked from outside branches which were fully exposed to sunlight. Kessler (1939) reported that for a given variety of apple those from Southern Germany were generally richer in vitamin C than those grown in the northern sections. Fruit which matured unevenly because of shading had from 30 to 50 per cent higher ascorbic acid content on the red than on the green side. The effect was attributed to the difference in light intensity rather than to an association with the red pigment. Burrell, Brown, and Ebright (1940) found the same variety of cabbage did not have the same ascorbic acid values at different seasons or in different regions. Lojander (1940) reported considerable seasonal variation in the vitamin C content of most garden vegetables. Illynview and Ulanova (1937) found that shortening the day decreased the relative quantities of ascorbic acid in kohlrabi and sorrel and increased the content in onions. Moldtmann (1939) observed that prolonged cloudiness lowered the ascorbic acid values in plants. He also determined the vitamin C content of leaves of *Fagus sylvatica* and of *Taxus baccata* at different times of year and found the highest values in *Fagus* in July and in *Taxus* in March. Sugawara (1939) studied the influence of variations in light intensity on the accumulation of ascorbic acid in various types of etiolated seedlings. Five-day-old seedlings of radish, Chinese cabbage, and maize grown in darkness were exposed to different intensities of light for 6, 18, 30, and 42 hours at 10,000, 1,000, 100, and 10 lux and others were left in darkness. After 42 hours exposure the following values (mg./g.

[1] Publication of the figures was assisted by the Lucien M. Underwood Memorial Fund.

fresh weight) in the above sequence were found: radish, 1.11, 0.95, 0.77, 0.67, 0.61; Chinese cabbage, 1.12, 1.02, 0.79, 0.63, and 0.56; maize, 0.67, 0.63, 0.56, 0.52, and 0.49. The ascorbic acid content is thus shown to increase with increasing light intensity but not in direct proportion to it. The effect of variations in the intensity of light upon the ascorbic acid values of excised *Ficus* branches placed in water and kept in darkness for eight hours previous to assaying was studied by Moldtmann (1939). He found a 20 per cent loss during the period in darkness and a 14 per cent gain as a result of a three-hour exposure to a 40 watt light at a distance of 30 centimeters. With successive increases in light intensity up to 600 watts higher ascorbic acid values were found but as in Sugawara's results the increase in ascorbic acid was not proportional to the increase in light intensity, particularly in the higher intensities.

Some investigators who have studied the effect of soil fertility upon the vitamin C content of plants have found that high fertility is favorable to its accumulation (Potter and Overholser 1933; Ijdo 1936; Hoffman, Krauss, and Washburn 1938; Fellers, Young, Isham, and Clague 1934). Others have observed comparatively little effect (Pfützer and Pfaff 1935; Smith and Paterson 1937; Smith and Gillies 1940). High nitrogen fertilizers have generally been found to influence ascorbic acid synthesis favorably, more than do other types of fertilizers (Isgur and Fellers 1937; Balks and Pommer 1938; Sugawara 1938; Burrell, Brown, and Ebright 1940). There is little reported evidence that the use of properly balanced fertilizers decreases the ascorbic acid content of plants grown under field conditions (Virtanen 1936; Isgur and Fellers 1937). Kessler, however, found (1939) that overfertilization of apple trees with nitrogen depressed the ascorbic acid content of the fruit and suggested that it was possibly caused by the shading of the fruit with the extra-heavy foliage of the high-nitrogen trees. Fuhrmeister (1937) on the contrary had previously obtained similar results with high-nitrogen fertilization of vegetables in which shading was supposedly not an interfering factor.

Moldtmann (1939) published data suggesting a parallelism between the glucose and ascorbic acid content of leaves of monocots but not of dicots. Other experiments by this investigator showed that within certain limits increasing the available carbon dioxide resulted in an increased synthesis of ascorbic acid. On the other hand, ringing experiments, which normally lead to an increase in carbohydrates above the ring, resulted in no increase in ascorbic acid content. Leaves above a ring in *Fraxinus excelsior* had definitely less ascorbic acid than similar leaves from normal branches. No distinct difference was found in tests with *Salix capra*.

The inter-relationships of available nitrogen, carbohydrates, and growth were studied by Kraus and Kraybill (1918), Nightingale (1922), Nightingale

and Kraus (1924), Reid (1924, 1929), and by a number of other investigators. It was found that the accumulation of carbohydrates is dependent upon the quantity of available nitrogen. If the nitrogen supply is very abundant, the carbohydrates tend to be utilized in growth and in synthesis of organic nitrogen compounds. On the other hand, if the supply of nitrogen is limited, carbohydrate utilization is retarded, and a marked accumulation may result.

No investigations have been reported bearing on ascorbic acid as influenced by the interrelations of mineral nutrient supply with seasonal and weather variations in light. The present studies are concerned with some aspects of these problems.

PROCEDURE

Approximately uniform cowpea seeds of the Groit variety were placed in germinators and kept at 27–29° C. for 24 hours or slightly longer. Seedlings with sprouts approximately 1 cm. long were selected for planting in clean white sand in half-gallon crocks of the shallow type. A nutrient solution was added under the conditions indicated for individual tests.

In preparing plants for vitamin C assays, the organs were weighed rapidly, then placed in Petri dishes and kept in the refrigerator until the ascorbic acid determinations by the indophenol method could be made. The vitamin C assays were made as rapidly as possible and if there was a fairly large number of samples, tests of the leaves were always made first and of the stems last. The tissues were pulverized in a mortar using a small quantity of acid-washed sand and 5 per cent metaphosphoric acid as an extracting agent. Appropriate aliquots of the filtered extracts were used in the titrations.

EXPERIMENTAL RESULTS

1. **Effect of Seasonal Differences in Light Conditions.** Two groups of young seedlings grown without added nutrients and at different seasons of the year were assayed in the first test. Some were grown during the latter part of November under the comparatively weak illumination and short days characteristic of this season and others in the much higher light intensity and longer days of March. The records of the U. S. Weather Bureau for Washington, D. C., show that the average total amount of solar radiation per day during the period of the November test was 184 gram-calories and in the March test, 344 gram-calories. The light intensity on bright days during the November test as measured in the greenhouse by a Weston illumination meter was about 60 per cent of that on bright days in March. The plants grown during November in the weaker light had larger stems and smaller roots than those grown in March.

TABLE 1. *Comparison of total ascorbic acid values and green weights of 14-day-old cowpea plants grown in November and March*

	Nov. 20–Dec. 1 Average radiation (Gr.-cal. per day) 184		Mar. 7–17 Average radiation (Gr.-cal. per day) 344		Nov./Mar. Radiation ratio 54		
	Green Wt. G.	Asc. Ac. Mg.	Green Wt. G.	Asc. Ac. Mg.	Green Wt.	Asc. Ac.	
						Total	Mg./G.
Leaves	0.796	0.525	0.851	0.880	0.94	0.60	0.64
Stems + Petioles	0.532	0.072	0.390	0.079	1.36	0.91	0.67
Roots	1.256	0.210	1.642	0.325	0.77	0.65	0.84
Total	2.584	0.807	2.883	1.284	0.90	0.63	0.70

Table 1 shows that the ascorbic acid values were higher in all parts of the March plants. The total ascorbic acid in the November plants was 63 per cent of that of the March plants; whereas, the total green weight of the former was only 11 per cent less than that of the latter. As calculated from the quantitative data in table 1, the leaves of the November plants contained 0.66 mg. of ascorbic acid per gram whereas those of the March plants contained 1.03 mg. per gram. The iodine and Flückiger tests[2] made upon sections of leaf and stem tissues showed a lower content of starch and free-reducing substances respectively in the November than in the March plants. The larger leaves, longer stems, smaller root systems, and lower content of starch and free-reducing substances found in these tests to be characteristic of cowpea plants grown under low light intensity and short days as compared with those grown under high illumination and long days are much like those previously described for Hubbard squash plants grown under similar conditions (Reid 1930).

In another experiment winter- and spring-cultured plants supplied with added nutrients were allowed to grow for eight weeks before being assayed. The winter group was tested on January 12 and the spring-summer group on May 19. The U. S. Weather Bureau records show that the average total radiation per day during the period in which the winter plants were grown was 165 gram-calories and during the spring-summer period was 470. The total radiation per day during the former period was only 35 per cent of that in the latter. However, during the week immediately preceding the winter test, the average total radiation per day was 41 per cent of that during the spring period. Measurements made in the greenhouse with the illumination meter showed almost a 50 per cent difference in the maximum intensities at the two seasons. The results of the experiment are shown in table 2.

[2] Eckerson, *Outlines of Plant Microchemistry* (unpublished).

TABLE 2. *Total ascorbic acid content and green weights of plants eight weeks old and tested in January and May*

	Nov. 17–Jan. 12 Average radiation 165 gr.-cal. per day		Mar. 24–May 19 Average radiation 470 gr.-cal. per day		Nov.–Jan./Mar.–May Radiation ratio 36		
	Green Wt. G.	Asc. Ac. Mg.	Green Wt. G.	Asc. Ac. Mg.	Green Wt.	Asc. Ac.	
						Total	Mg./G.
Leaves	18.99	9.85	18.44	20.44	1.03	0.48	0.47
Stems + Petioles	15.66	1.17	13.03	1.64	1.20	0.71	0.60
Roots	9.36	1.07	17.23	2.34	0.54	0.46	0.84
Total	44.01	12.09	48.70	24.42	0.90	0.50	0.55

The winter plants accumulated half as much ascorbic acid as the spring-summer plants. The relative difference in size of the root systems is greater than that observed in the younger plants of the first test. A pronounced difference in the total and percentage ascorbic acid values and only a slight variation in green weight in the leaves of the two groups of plants is to be noted.

Some of the plants in the November-December test were grown under a cheesecloth screen which reduced the light intensity about 50 per cent. On January 12, when the plants were 8 weeks old, tests were made to determine the ascorbic acid values of successive leaves of the shaded and unshaded plants. The data thus obtained are shown in table 3 together with the results of similar assays of the leaves of the plants tested on May 19.

The leaves of the winter plants contained 57 per cent as much ascorbic acid expressed as absolute amount and 48 per cent as much on a green

TABLE 3. *Ascorbic acid content of leaves of plants grown in normal and reduced intensity of daylight and at different seasons*

	Eight-week-old plants tested Jan. 12				Eight-week-old plants tested May 19	
	Plants grown in daylight of normal intensity		Plants grown in partial shade			
	Mg./Plt.	Mg./G.	Mg./Plt.	Mg./G.	Mg./Plt.	Mg./G.
First (oldest) leaf	0.47	0.46	0.30	0.39	0.43	0.91
Second " "	0.66	0.43	0.35	0.30	1.21	1.02
Third " "	1.11	0.47	0.51	0.32	1.99	1.04
Fourth " "	1.49	0.47	0.77	0.38	3.21	1.07
Fifth " "	1.69	0.51	0.93	0.38	3.26	1.10
Sixth " "	1.67	0.47	0.95	0.38	2.75	1.04
Seventh " "	1.60	0.49	0.97	0.37	2.86	1.16
Eighth " "	1.43	0.68	1.04	0.48	2.32	1.19
Ninth " "	0.83	0.94	0.55	0.63	1.10	1.66
Total	10.94	0.52	6.37	0.39	19.13	1.09

weight basis as did those of the spring-summer plants. Shading of the winter plants caused a reduction of approximately 42 per cent in total ascorbic acid and a 25 per cent lower value on a percentage green weight basis.

2. Effect of Varying Intensities of Light. Further studies were conducted to determine the effect of varying light caused by changing weather conditions on ascorbic acid accumulation. Twelve-day-old plants grown in normal daylight were assayed for ascorbic acid on April 4 at 2:00 p.m. At approximately the same time on the next day cultures from the following environments were assayed: normal light intensity, normal light reduced about 75 per cent by a cheesecloth screen, such partial shade cultures which had been transferred to full daylight 24 hours previously, and normal daylight cultures transferred to darkness 20 hours previously. As indicated in table 4, approximately similar procedures were followed on the next two days.

The plants grown under reduced light intensity contained only 40 per cent as much ascorbic acid on the basis of absolute amount as those grown in normal light and 59 per cent as much on a green weight basis. The greatest effect of the difference in light intensity was found in the leaves and least in the stems. Exposure of the shaded plants to normal daylight for twenty-four hours caused an increase in the total ascorbic acid, most of the increase being in the leaves. Exposure to normal daylight for two days following partial shading resulted in a 55 per cent higher value for the absolute amount than was found in similar plants left in the shade. At this time a 20 per cent higher total value in the roots and a 24 per cent higher total value in the stems was found in the more highly illuminated plants. Keeping the plants in darkness for twenty-four hours previous to assaying resulted in losses of the vitamin of approximately 15 per cent. The data indicate that all parts of the plant were involved in the decrease. Plants grown in daylight of normal intensity and transferred to the low intensity chamber forty-eight hours before assaying had an 11 per cent lower total ascorbic acid value on April 6 and others a 24 per cent lower value on April 7 than similar plants left in normal daylight. The lower value of the normal plants on April 6 was associated with very cloudy weather. Presumably at the time of assaying complete recovery from the metabolic loss of the previous night had not occurred because of the weak light (Reid 1941).

3. Comparative Effects of Differences in Light Intensity and Length of Day with and without Additional Nitrogen. An experiment was conducted in March in which environmental variations were employed, simulating in some respects the normal seasonal changes in light intensity and length of daily period of illumination. Thirty-six cultures of ten plants each were prepared, nine of which were placed under each of the four following experimental conditions:

TABLE 4. *Variations in growth and ascorbic acid content of young cowpea plants in relation to variations in conditions of illumination*

Conditions during growth		Green Wt. (g.)	Ascorbic Acid (mg.)	Ascorbic Acid (mg./g.)
		April 4—Very bright		
12 days normal daylight	Entire Plant	2.56	1.070	0.418
	Stems	0.37	0.081	0.215
	Roots	1.52	0.293	0.193
	Leaves	0.675	0.696	1.031
		April 5—Very bright		
13 days normal daylight	Entire Plant	2.78	1.280	0.460
	Stems	0.37	0.101	0.277
	Roots	1.68	0.347	0.253
	Leaves	0.728	0.832	1.143
12 days normal daylight, 1 day in darkness	Entire Plant	2.58	0.904	0.346
	Stems	0.37	0.069	0.187
	Roots	1.51	0.245	0.162
	Leaves	0.703	0.590	0.839
13 days weak daylight	Entire Plant	1.88	0.510	0.271
	Stems	0.45	0.068	0.151
	Roots	0.83	0.156	0.187
	Leaves	0.596	0.286	0.480
12 days weak daylight, 1 day normal daylight	Entire Plant	1.97	0.570	0.289
	Stems	0.49	0.073	0.150
	Roots	0.84	0.158	0.188
	Leaves	0.640	0.339	0.530
		April 6—Very cloudy		
14 days normal daylight	Entire Plant	2.66	1.079	0.406
	Stems	0.38	0.095	0.245
	Roots	1.60	0.314	0.196
	Leaves	0.678	0.670	0.988
12 days normal daylight, 2 days weak daylight	Entire Plant	2.79	0.818	0.258
	Stems	0.40	0.077	0.193
	Roots	1.70	0.291	0.171
	Leaves	0.692	0.450	0.650
14 days weak daylight	Entire Plant	2.24	0.456	0.203
	Stems	0.54	0.066	0.122
	Roots	1.00	0.169	0.168
	Leaves	0.696	0.222	0.319
12 days weak light, 2 days normal daylight[a]	Entire Plant	2.21	0.710	0.322
	Stems	0.55	0.082	0.148
	Roots	1.00	0.203	0.203
	Leaves	0.664	0.425	0.640

TABLE 4—(*Continued*)

Conditions during growth		Green Wt. (g.)	Ascorbic Acid (mg.)	Ascorbic Acid (mg./g.)
		April 7—Very bright		
15 days normal daylight	Entire Plant	2.92	1.104	0.365
	Stems	0.41	0.099	0.241
	Roots	1.81	0.345	0.191
	Leaves	0.700	0.660	0.943
15 days weak daylight	Entire Plant	2.39	0.533	0.223
	Stems	0.55	0.068	0.124
	Roots	1.16	0.175	0.155
	Leaves	0.678	0.290	0.428
13 days normal daylight, 2 days weak daylight	Entire Plant	2.90	0.978	0.339
	Stems	0.41	0.108	0.263
	Roots	1.82	0.307	0.168
	Leaves	0.676	0.563	0.833
14 days normal daylight, 1 day darkness	Entire Plant	2.75	0.924	0.336
	Stems	0.38	0.081	0.213
	Roots	1.69	0.300	0.176
	Leaves	0.676	0.543	0.803

[a] Last day was very cloudy.

1. Normal light intensity and normal length of day.

2. Short day. Daily illumination period of seven hours. Frame covered with black sateen placed over plants at 4:00 p.m. and removed at 9 a.m.

3. Long Day. Daily illumination period of seventeen hours. 150 watt Mazda lights placed 30 inches above the plants were on from 5:00 to 11:00 p.m.

4. Partial shade. Plants were placed under a frame covered with cheesecloth which reduced the normal light intensity in the greenhouse by about 65 per cent.

On the tenth day and each day thereafter a complete nutrient[3] solution was applied. The ascorbic acid assays were begun on the third day when the primary leaves were emerging above the surface of the sand. Roots, stems, and leaves were analyzed separately. Leaf areas were also determined. Plants grown under the different environmental conditions were prepared for the tests at 11:00 a.m.

Reducing the light intensity during the month of March had a much more marked effect upon ascorbic acid values than did lengthening or shortening the daily period of illumination. During the time of partial dependence of the seedlings upon the stored food reserves in the cotyledons,

[3] The mineral elements were present in the following concentrations (p.p.m.): N = 90, P = 45, K = 75, Ca = 40, Mg = 20, S = 36, Cl = 58, Fe = 2.5, Mn = 0.1, B = 0.1, Zn = 0.1.

that is up to and including the fifth day, variations in light intensity or in length of the daily period of illumination had less influence than after the plants became more nearly independent. The total ascorbic acid per plant at successive stages of development under each of the four environmental conditions is shown in figure 1 and the green weights of the plants in figure 2.

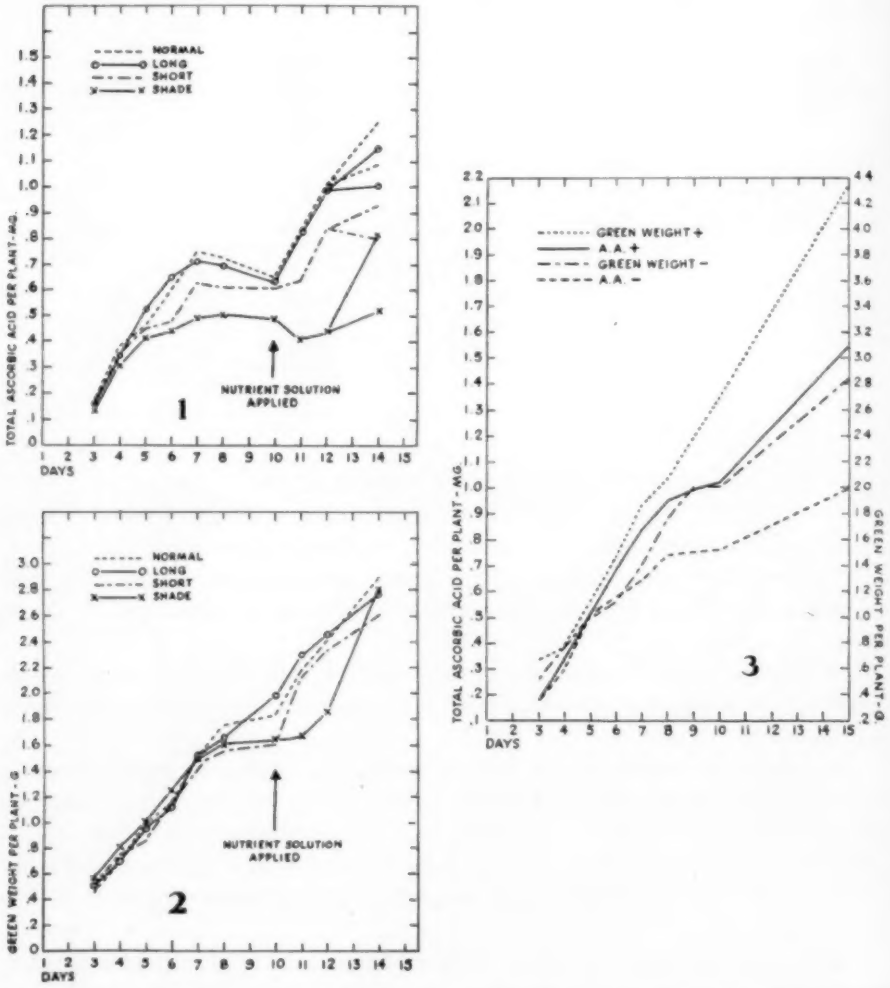

FIG. 1. Total ascorbic acid content of young cowpea plants grown under different conditions of illumination. The lower points on the curve on the fourteenth day represent the ascorbic acid values exclusive of the compound leaves and the upper points the values with the compound leaves included. FIG. 2. Green weights of young cowpea plants grown under different conditions of illumination. FIG. 3. Green weights and total ascorbic acid in young plants, some grown with (+) and some without (−) added nutrients.

From the seventh to the tenth days inclusive there was a lag in ascorbic acid accumulation under each of the four conditions of illumination. There was also a decrease in the rate of growth apparent on the seventh or eighth days, continuing to the tenth day. A pronounced lag in growth and ascorbic acid accumulation at this stage of development has also been noted in previous experiments in plants grown without added nutrients and a slight retardation in ascorbic acid increase even in well fertilized plants (Reid 1941).

The (total) ascorbic acid of the short-day and partially shaded plants remained practically constant from the seventh to the tenth days; whereas, the results suggest a loss from the normal and long-day plants on the tenth day (Reid 1941). No determinations were made on the ninth day. The apparent losses followed two days of very cloudy weather. There is a suggestion here, as there also has been in previous tests, that metabolic losses of ascorbic acid during periods of darkness are greatest in plants which have been most active photosynthetically during the immediately preceding periods of illumination.

Microchemical examination of the tissues revealed that the normal- and long-day plants had accumulated considerable starch and free-reducing substances, those of the short-day plants somewhat less, and those of the shaded plants relatively little. The internal symptoms of the normal- and long-day plants suggested that a state of nitrogen deficiency was developing. On the eleventh day, following the application of nutrient solution on the tenth day, a definitely higher ascorbic acid content and a stimulation of growth were found in these plants. An appreciable gain in ascorbic acid was not evident until a day later in the short-day plants. The addition of nutrients appeared to cause a depression in the total amount of ascorbic acid in the shaded plants for two days immediately following application of the solution. Between the twelfth and fourteenth days, however, a very rapid gain occurred. This was caused chiefly by the rapid development of the first set of compound leaves. The ascorbic acid values on the fourteenth day, with and without inclusion of the compound leaves, may be observed in figure 1, the lower points on the fourteenth day representing the values without the compound leaves and the upper points the values with the compound leaves included. A more rapid development of the first set of compound leaves was observed in the shaded plants than in the other groups. This tendency toward a somewhat more rapid initiation and development of leaves has been noted previously in plants grown in light but under conditions allowing a relatively slow rate of carbohydrate synthesis (Reid 1930).

In other tests conducted at a time of year when light conditions were similar to those of the preceding test no nutrients were added but nodules were allowed to develop on the roots. The apparent availability of nitrogen

was soon reflected in an increase both in growth and in total ascorbic acid similar to the responses to nutrient solution. The results of one of these tests are shown in figure 3. The plants which had received nutrient solution since the third day (the time of emergence above the sand) were much larger on the fifteenth day than the plants which received no nutrient solution. No assays were made between the tenth and fifteenth days. Doubtless, if daily determinations of ascorbic acid in the plants grown without added nutrients had been made during this period, the curve would have continued in the horizontal direction for some time after the tenth day and, as nitrogen became available, would then have risen more steeply than is represented in the graph. The results of this experiment suggest that the stimulatory effect on growth and ascorbic acid synthesis noted after application of a complete nutrient solution to very young plants is caused chiefly by the added nitrogen. The addition of nutrient solution lacking in other elements[4] such as phosphorus, potassium, calcium, and magnesium have not produced definite differences in the total ascorbic acid in plants at such an early stage of development. Apparently these elements become limiting factors both in growth and in ascorbic acid synthesis at later stages than does nitrogen.

Reducing the light intensity resulted in a greater spread of the leaf blade which became evident as early as the fifth day, later becoming more pronounced. There were, however, no marked differences in the green weights of leaves of the four groups of plants. The greater thickness of the leaves of the plants grown in normal daylight as compared with the leaves of the shaded plants compensated in weight for the smaller areas of blades of the former. On the fourteenth day the primary leaves of plants grown in daylight of normal intensity both under normal- and long-day conditions contained 0.019 mg. of ascorbic acid per square centimeter of leaf surface whereas those of the shaded plants contained only 0.007 mg. per square centimeter and those of the short-day plants 0.016 mg. The thicker leaves of the plants grown in daylight of normal intensity contained a larger number of chloroplasts per unit area of tissue than the thin leaves of the shaded plants, a difference which undoubtedly has a determining influence upon the ascorbic acid concentration per unit area of mesophyll surface. There is considerable evidence that ascorbic acid synthesis is associated with the functioning of the chloroplasts (Geitler 1922; Girond, Leblond, and Ratsimanga 1934; Gautheret 1935; Wieler 1936; Weber 1936; Dischendorfer 1937; Pekarek 1938; Weier 1938; Bukatsch 1940). Tests made with very young cowpea leaves, before there is much development of the mesophyll, have shown that the ascorbic acid content is about the same as that of the young portion of the stem; but with development of the mesophyll under favorable light conditions there is an increase of several hundred per cent

[4] Unpublished data.

per unit of weight. For example, young leaves from the seventh node which were enclosed within bud scales but with tips emerging yielded values of 0.22 mg. per gram; whereas those at the sixth node which were expanding after emergence from the bud scales contained 1.31 mg. per gram.

The partially shaded plants of the present experiment had longer, heavier stems than those of the other three groups grown in daylight of normal intensity, but the ascorbic acid values tended to be somewhat lower, the differences increasing as the plants became older. Toward the end of the experimental period, when the first set of compound leaves was developing, the differences were pronounced. Somewhat lower values were observable also in the short-day as compared with the normal and long-day plants in the latter portion of the test period.

The ascorbic acid values for the roots of the different groups of plants varied in the same directions as did those of the stems, low intensity being the only one of the four conditions causing marked differences. For example, the roots of normal-, short-, and long-day plants respectively contained 0.34, 0.25, and 0.31 mg. of ascorbic acid on the fourteenth day, whereas those of the shaded plants contained only 0.13 mg.

TABLE 5. *Summary of results in the preceding experiments on the relation of light intensity to ascorbid acid accumulation*

Maximum Light Intensities (approximate values in f. c.)		Experiment	1	2	3	4	5
	2500	Total (Mg. per plant)				0.510	
		Mg./G. in leaves			0.390	0.403	
	3500	Total (Mg. per plant)					0.420[a]
		Mg./G. in leaves					0.480
	5000	Total (Mg. per plant)					
		Mg./G. in leaves		0.519	0.520		
	6000	Total (Mg. per plant)	0.807				
		Mg./G. in leaves	0.660				
	9000–10,000	Total (Mg. per plant)	1.284			1.280	1.090[a]
		Mg./G. in leaves	1.034	1.108	1.090	1.015	1.143

[a] These values are somewhat low, because the nutrient solution was not added until the plants were ten days old. Total values in experiments 2 and 3 cannot be compared with the others because the plants were much older.

Table 5 shows the ascorbic acid content (mg./g.) of the leaves of the plants employed in the previous experiments for which different light intensities prevailed. The values for the latter represent approximately the maximum intensities found during the course of the experiments. A comparison of the total quantity of vitamin C of plants grown under different light intensities cannot be made in experiments 2 and 3 because the plants were eight weeks old as compared with fourteen- and fifteen-day-old plants in the other three tests. A relation between light intensity and both absolute and percentage content of ascorbic acid is clearly indicated but further tests using more refined methods are required to determine exact quantitative relationships.

DISCUSSION

The data herein presented afford an insight into some of the more common causes of variation in the vitamin C content of green plants. The differences in light factors characteristic of different seasonal and weather conditions are of importance. It should be emphasized that these results were obtained chiefly with young seedlings which are known to be very sensitive to differences in light intensity. It is quite possible that the length of day factor would have a greater influence in older cowpea plants or in other types of plants. An investigation of the ascorbic acid relations in plants whose reproductive period is determined by length of day would be of special interest. It is recognized that reducing the average light intensity of plants grown in March, April, and May to approximate the average intensity in December by the methods herein employed afford at best only a crude approach toward natural climatic conditions.

The present results are in general agreement with those of Sugawara and Moldtmann in showing that the ascorbic acid content of plants increases with increasing light intensity and they also suggest that the relationship may not be linear. Sugawara and Moldtmann have shown that differences in illumination at the lower intensities produce relatively greater differences in ascorbic acid synthesis than similar differences in the higher intensities. This is also the now generally accepted view of the relation between variations in light intensity and the rate of carbohydrate synthesis.

The question of the relative importance of light intensity and the total amount of light in influencing ascorbic acid synthesis is still unsettled. Light intensity is of great importance, in part, because of its effect upon the structural development of the leaf with respect to the organization and depth of the palisade tissue, thereby affecting the number and distribution of the chloroplasts. It may also be essential for the synthesis of a protein-like constituent contained within the latter. However, we do not yet know whether its chief importance consists in the formation of these structural elements or whether it is mainly concerned in their functioning. If it is

important in connection with the former, then a time factor in ascorbic acid synthesis may be of major importance. For example, an etiolated seedling placed in darkness as in Sugawara's experiments would tend to function slowly in ascorbic acid synthesis until an increase occurred in some other constituent, supposedly originating in the chloroplasts.

The results of the present tests and those of other workers suggest the possibility of a functional relationship between the synthesis of ascorbic acid and that of carbohydrates. It is possible that the product of one of these two types of synthesis may result from that of the other but the question remains as to which, if either, takes precedence. It is possible, on the other hand, that these substances may be simultaneous products of chloroplastic activity.

Previous studies (Reid 1930, II) have shown the importance of light and the necessity of a supply of nitrogen for the synthesis and maintenance of a plasmatic constituent of the chloroplasts. Since evidence suggests that the chloroplasts are the structures mainly concerned in the formation of ascorbic acid, an increased rate of synthesis when nitrogen is supplied to nitrogen-depleted plants is not surprising.

The data suggest that if a plant is grown under light conditions which permit a rapid synthesis of carbohydrates, ascorbic acid synthesis will be rapid also. If there is a deficiency of nitrogen, carbohydrates tend to accumulate, not so much because more are produced as because utilization is retarded. Under these conditions ascorbic acid may increase very slowly at first, and then will cease to accumulate as the deficiency becomes more pronounced. The evidence so far is inconclusive as to whether an abundance of nitrogen during periods of limited illumination is a depressing factor for the content of ascorbic acid as it is known to be for carbohydrates. At least it seems clear that high soil fertility with reference to nitrogen is conducive to vitamin C production only when light conditions allow synthesis of an ample supply of carbohydrates. On the basis of Fuhrmeister's (1937) and Kessler's (1939) work a very high nitrogen supply may actually be detrimental. Further study is required to determine conclusively to what extent such harmful effects may be overcome by increasing the amount of light.

These results suggest that weather conditions should be considered in determining the time for harvesting fruits and vegetables. Other experiments which will be described in detail elsewhere have shown that time of day is also important, especially for vegetables of leafy types. Consideration of these factors may be of special value in timing the selection of material for canning.

SUMMARY

High daylight intensity and medium to long days are conducive both to high absolute and high percentage ascorbic acid values in cowpea plants; whereas low intensity and short days have a depressing effect.

Seasonal variations, attributable to differences in conditions of illumination were found in the absolute and percentage content of ascorbic acid.

With differences in light intensity of 65 per cent and in length of the illumination period with normal daylight of 42 per cent, light intensity was found to have a greater influence than length of day. Lengthening the day with weak light (Mazda) to 17 hours so as to produce an 80 per cent increase in day length did not produce increases as great as a 65 per cent increase in light intensity.

Shifting ascorbic acid values were found as a result of environmental alterations such as occur under changing weather conditions. The variations are noted first in the leaves and somewhat later in the roots.

A sufficient supply of available nutrients with special reference to nitrogen is also conducive to high ascorbic acid values, particularly as to the total quantity, provided light conditions are such as to permit the synthesis of an ample supply of carbohydrates. It is suggested that the special relation of nitrogen to ascorbic acid formation in a green plant is dependent upon the necessity of this element for the synthesis and maintenance of the plasma content of the chloroplasts.

It is suggested that weather conditions involving varying intensities of light should be considered in determining the time for harvesting fruits and vegetables.

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THE RELATION BETWEEN XYLEM THICKENINGS IN PRIMARY ROOTS OF VICIA FABA SEEDLINGS AND ELONGATION, AS SHOWN BY SOFT X-RAY IRRADIATION

G. F. SMITH AND H. KERSTEN[1]

Previous studies on the root structure of *Vicia faba* seedlings grown from x-rayed seeds reported modifications in the secondary wall thickenings of the xylem elements (6). One observation indicated an alteration in the normal sequence of secondary wall thickenings including the annular, spiral, scalariform and pitted types. In the roots of these treated seedlings, very prominent pitted vessels occurred slightly above the apical meristem with only an occasional spiral or scalariform element in some specimens.

Morphology texts such as those of Jeffrey (2) and Haberlandt (1) discuss the relationship between these vessel types and the extent of elongation in the stem or root. A more complete study of this point is given in an early work of Stover (7) on the vascular anatomy of *Calamovilfa longifolia*. In it he maintains that the vessel types are the result of elongation. His observations indicate that annular and spiral xylem elements, which are rarely present in the rhizome of *Calamovilfa* because of the failure of internodal elongation, are present in the rapidly elongating aerial stems, as are also the later formed reticulate and pitted elements.

It is further implied by Stover, that the first thickening of a desmogen cell is of the pitted type, being laid down in the arrangement of a pitted cytoplasmic cell lining, the latter being a result of a cellular vacuolation which occurs early in the development of young cells of desmogen strands. After the formation of the original pitted thickening, division and elongation of surrounding parenchymatous cells causes it to be torn apart and to form annular, spiral, scalariform, or pitted arrangements, depending upon the extent of stretching. Consequently a pitted vessel of the metaxylem remains as such if the surrounding cells are no longer dividing or enlarging.

If the extent of elongation, therefore, determines the type of wall thickening of the xylem elements, variations in both of these factors in the roots of seedlings germinated from x-rayed seeds ought to show some relationship. On this basis, together with the fact that, within limits, the extent of elongation of the primary roots of plants grown from x-ray treated seeds decreases as the dose of irradiation increases, it seemed likely that a study of a series of *Vicia faba* primary roots with successively less elongation correlated with increasing x-ray irradiation might contribute information on

[1] The cost of publication of the figures was borne in part by the Department of Physics of the University of Cincinnati.

the relationship existing between the types of vessel secondary wall thickening and total elongation.

METHODS

The soft x-ray apparatus used here and the general character of its radiation have been described previously (3, 4). Dry *Vicia faba* seeds were placed with their hila toward the source of radiation in small glass dishes 8 cm. from the focal spot of the x-ray tube. The tube was operated at 30 peak kv., and 10 ma. Exposure times included 0, 15, 30, 60, 90, and 120 minutes.

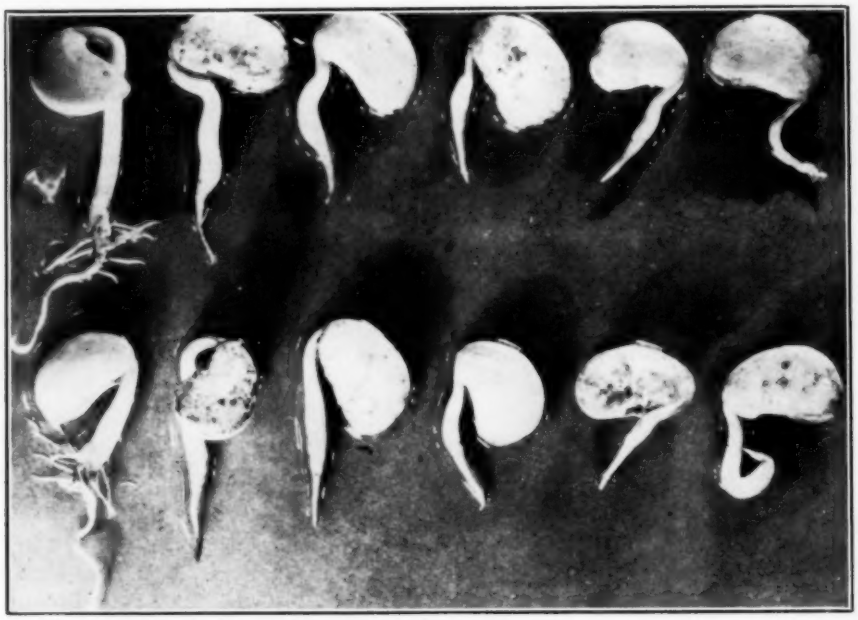

FIG. 1. Decreasing root lengths of 5-day-old *Vicia faba* seedlings grown from seeds x-ray irradiated at 30 kv., and 10 ma. for 0, 15, 30, 60, 90, and 120 minutes.

After irradiation, the seeds were soaked for 5 hours and later germinated in moist peat moss at 25° C. Material for histological studies was collected after five days, when the seedlings germinated from the seeds exposed for 120 minutes displayed the characteristic cessation of growth associated with the "delayed killing" effect in seedlings from x-ray irradiated seeds, as described by Maxwell (5). Primary roots of 50 seedlings of each experimental lot were prepared for microscopical study with standard methods of microtechnique.

In studying the histological developments in seedlings grown from x-ray treated seeds, it is necessary to prepare and observe a great quantity of sections because of the resulting irregularity in arrangement and formation

of tissues. Consequently the longitudinal sections used as illustrations in this paper are not medial throughout their entire extent, but they approach this position as nearly as possible, and the developments indicated represent data from several hundred slides.

OBSERVATIONS

Figure 1 indicates the extent of primary root elongation in seedlings grown from seeds which had been exposed to soft x-rays for periods of 0, 15, 30, 60, 90, and 120 minutes. The characteristic decrease in root length with increasing doses of x-ray irradiation is shown. The average primary root lengths, including the hypocotyl, of seedlings of each lot, are listed in Table 1.

TABLE 1

	Time of exposure to x-rays in minutes					
	0	15	30	60	90	120
Average length of primary root in centimeters	8.0	5.8	5.0	4.0	3.6	3.0

The failure of lateral root formation related to x-ray treatments of seeds is also observed. A discussion of this has already been given (6).

Medial longitudinal sections of the root tips of these seedlings, showing types of secondary wall thickenings of the xylem elements, are illustrated in figures 2–7. Figure 2 shows the occurrence of the vessel elements and the order of their formation in normal primary roots of *Vicia faba* seedlings. The relative range of prominence of each vessel type, as it appears in the roots of the seedling groups studied, is diagrammed in figure 8.

In examining longitudinal root sections of the seedlings germinated from x-ray irradiated seeds, the most evident modification of the normal arrangement is the appearance of large pitted vessels in the most distal part; vessels of other types are found in the earlier formed regions above (figs. 3–7). If pitted vessels are associated with the least elongation of the structure in which they are forming, as is suggested by Stover, and if types formed before these indicate more extensive elongation, one is led to assume that the appearance of elements related to least elongation during later development indicates a decline in growth so far as elongation is involved.

This implication becomes plausible when one considers the phenomenon of "delayed killing" or growth cessation (5) which occurs in seedlings from seeds given a medium dose of x-rays. After an apparently normal onset of germination, inasmuch as the radicles appear beyond the cotyledons at approximately the same time in both control and treated seedlings, there is a progressive decline in rate of elongation resulting ultimately in death. The

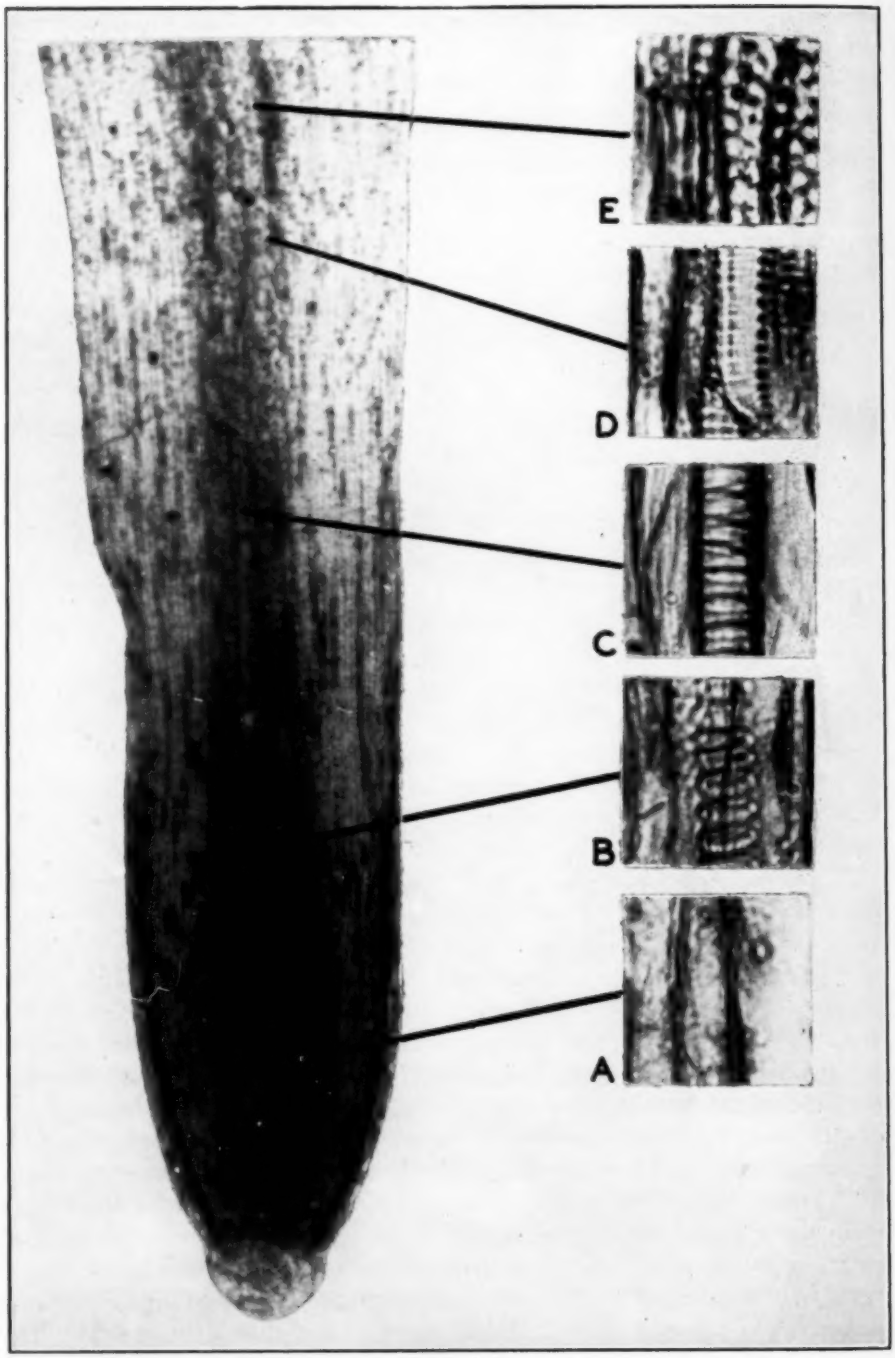

FIG. 2. Medial longitudinal root tip section of a normal *Vicia faba* seedling showing the occurrence of vessel types. × 40 and 675.

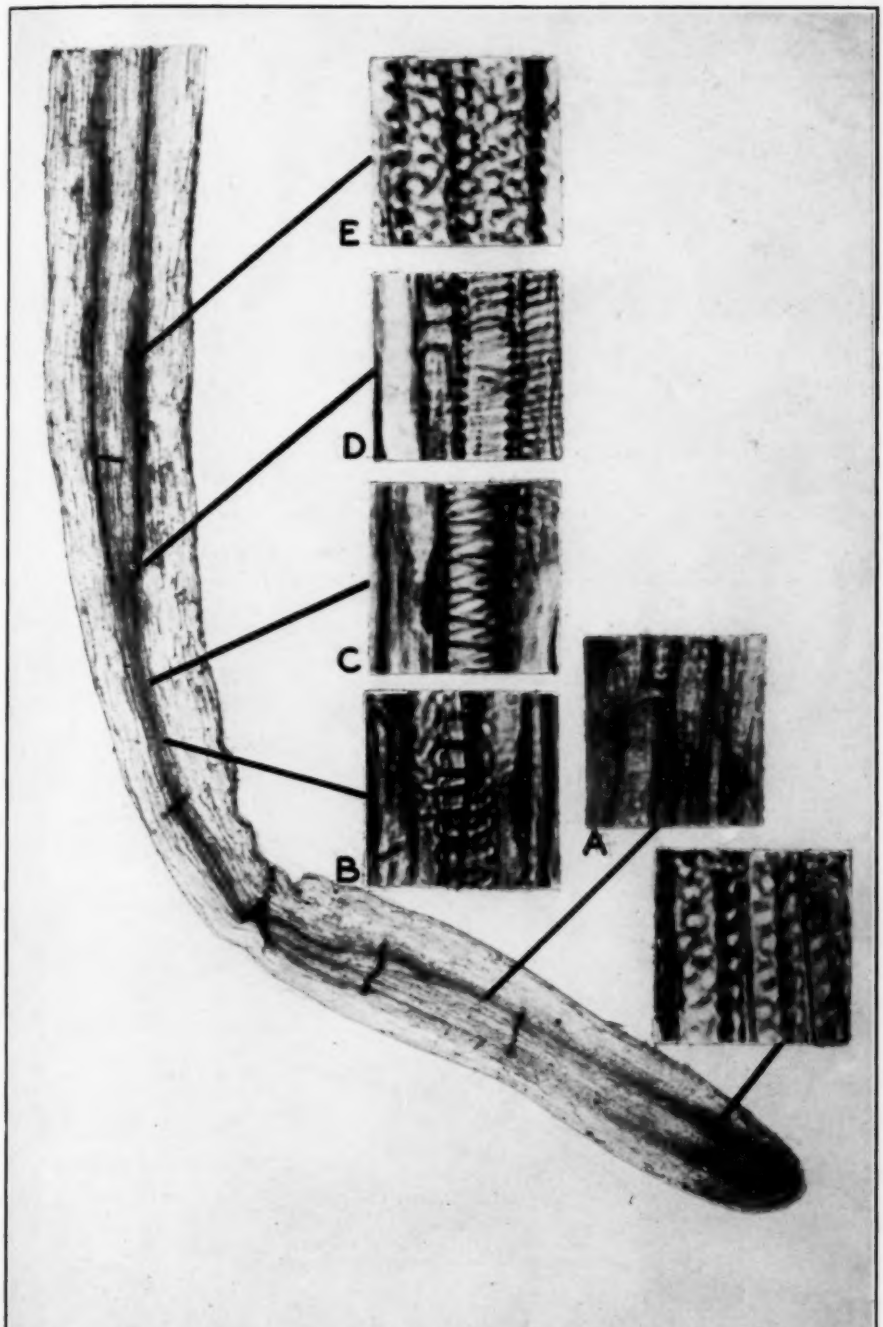

FIG. 3. Medial longitudinal root tip section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 15 minutes. × 20 and 675.

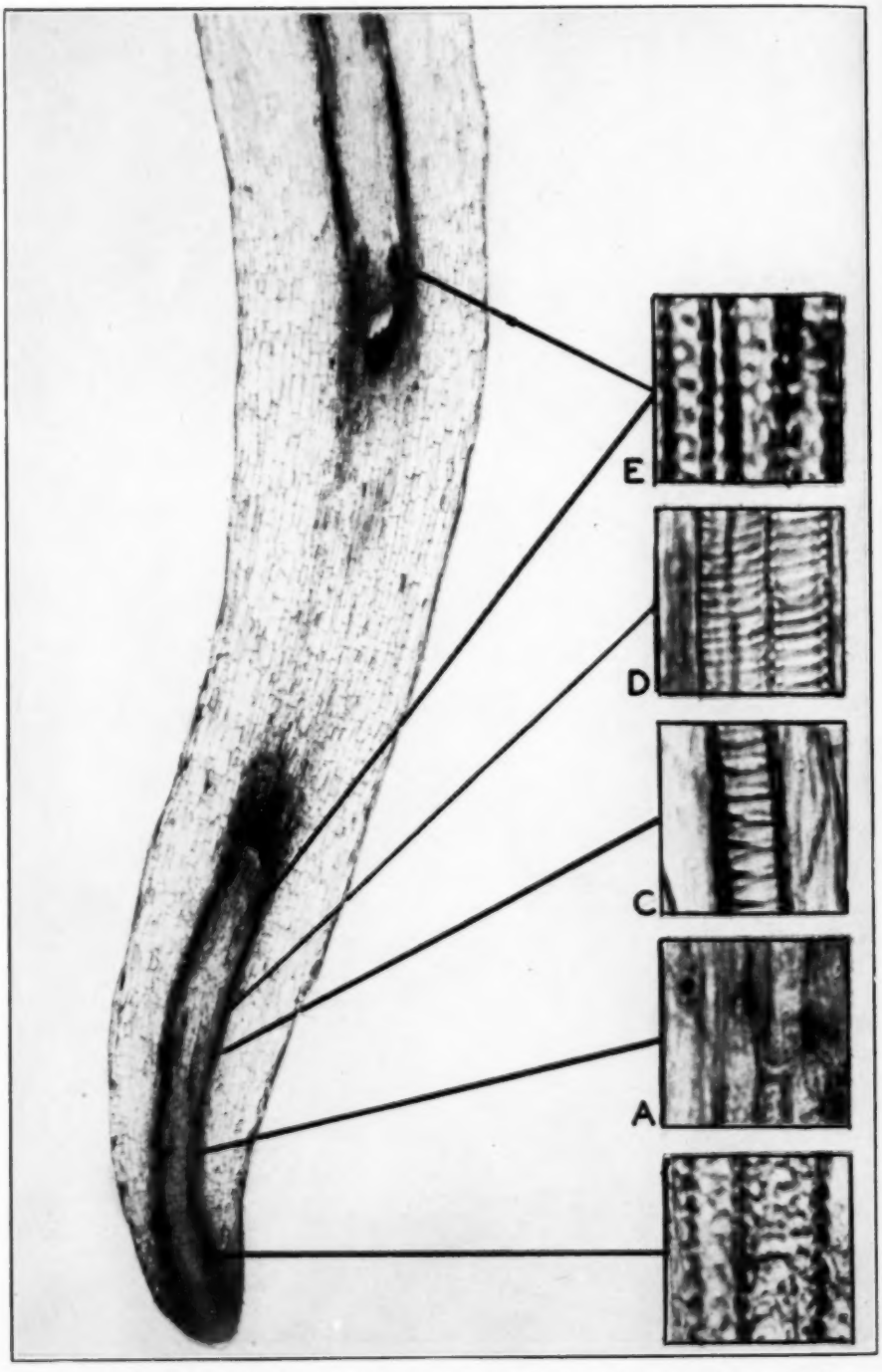

FIG. 4. Medial longitudinal root tip section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 30 minutes. × 20 and 675.

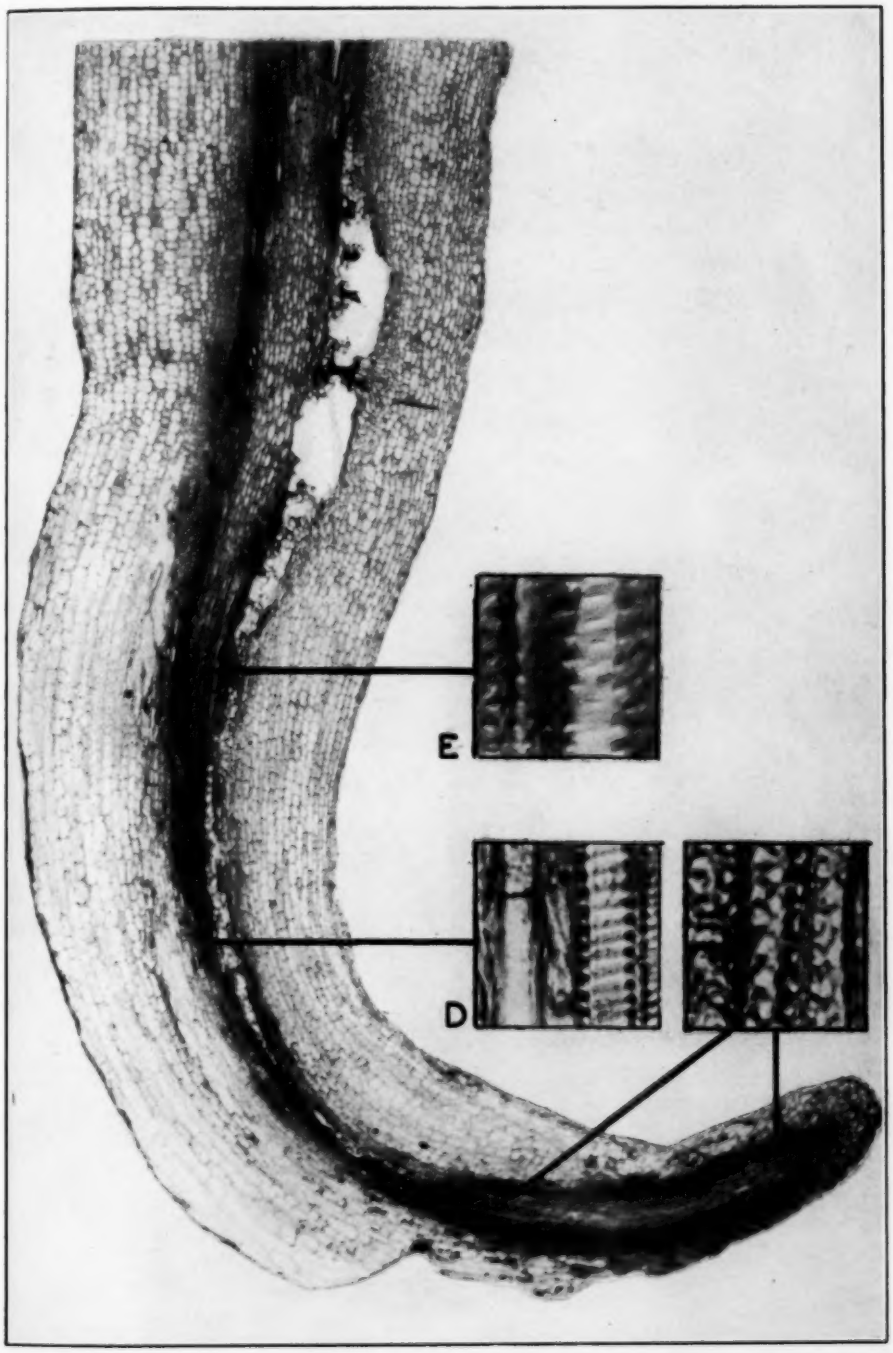

FIG. 5. Medial longitudinal root section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 60 minutes. ×20 and 675.

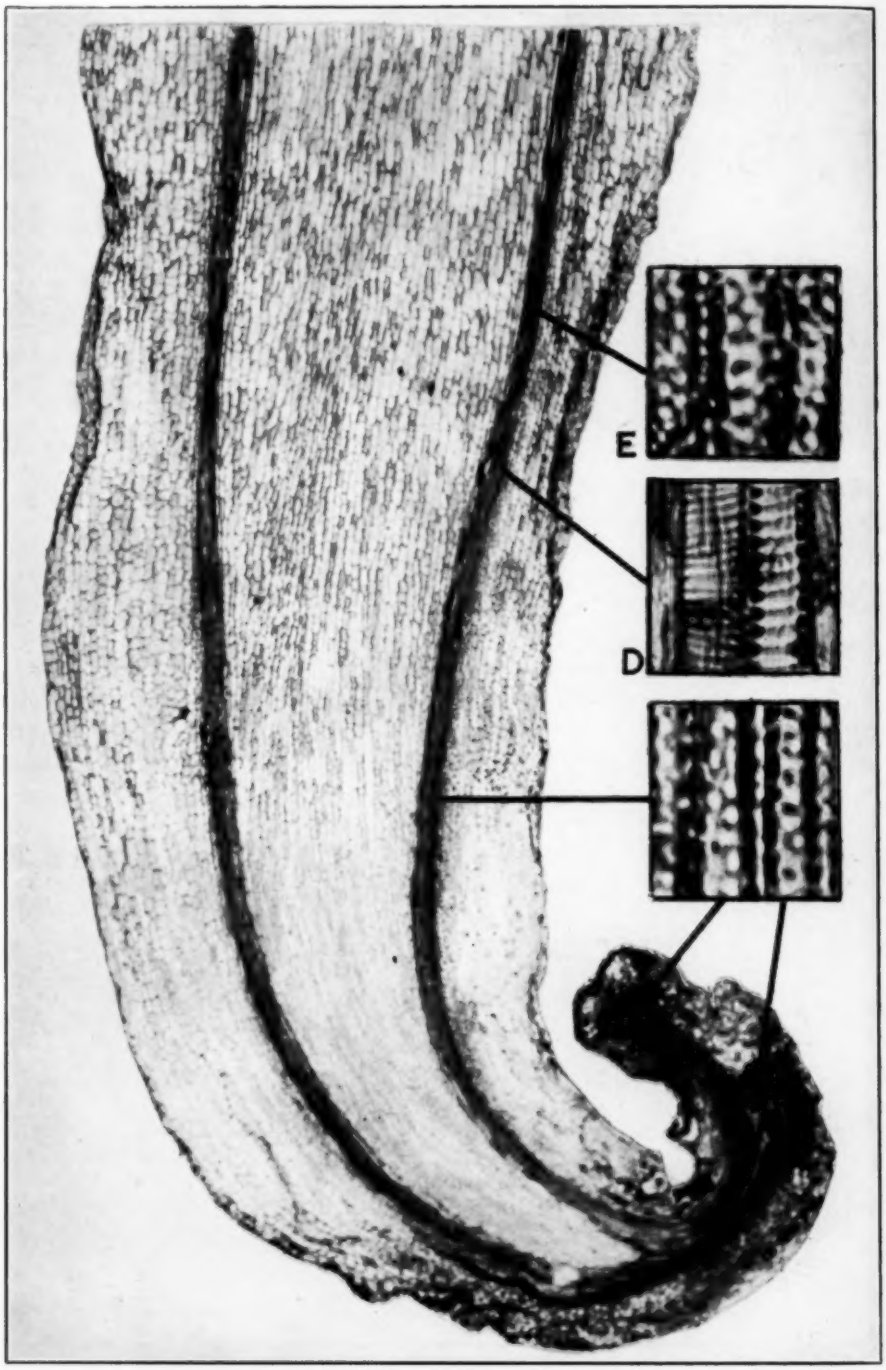

FIG. 6. Medial longitudinal root tip section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 90 minutes. × 20 and 675.

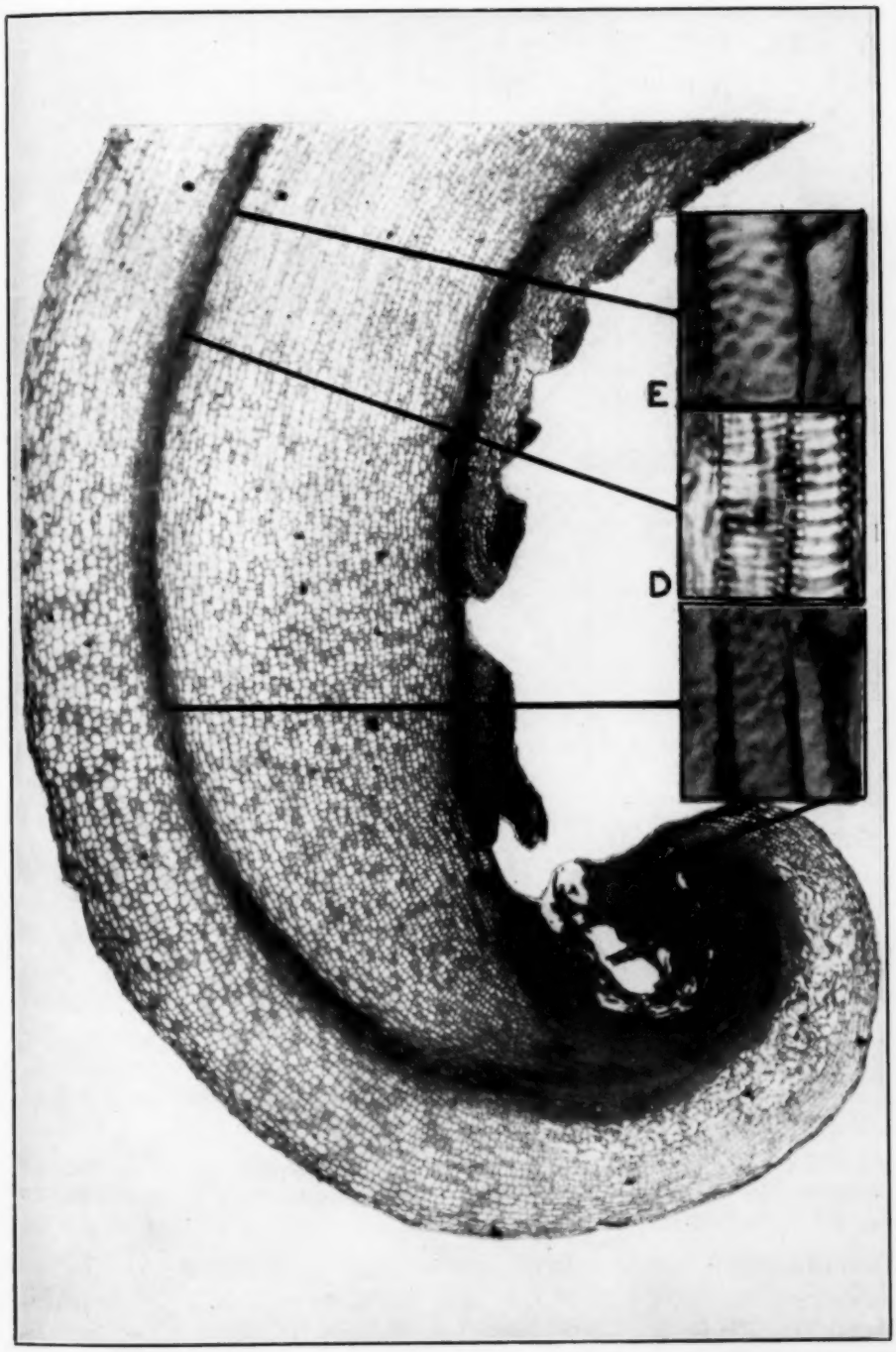

FIG. 7. Medial longitudinal root tip section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 120 minutes. × 20 and 675.

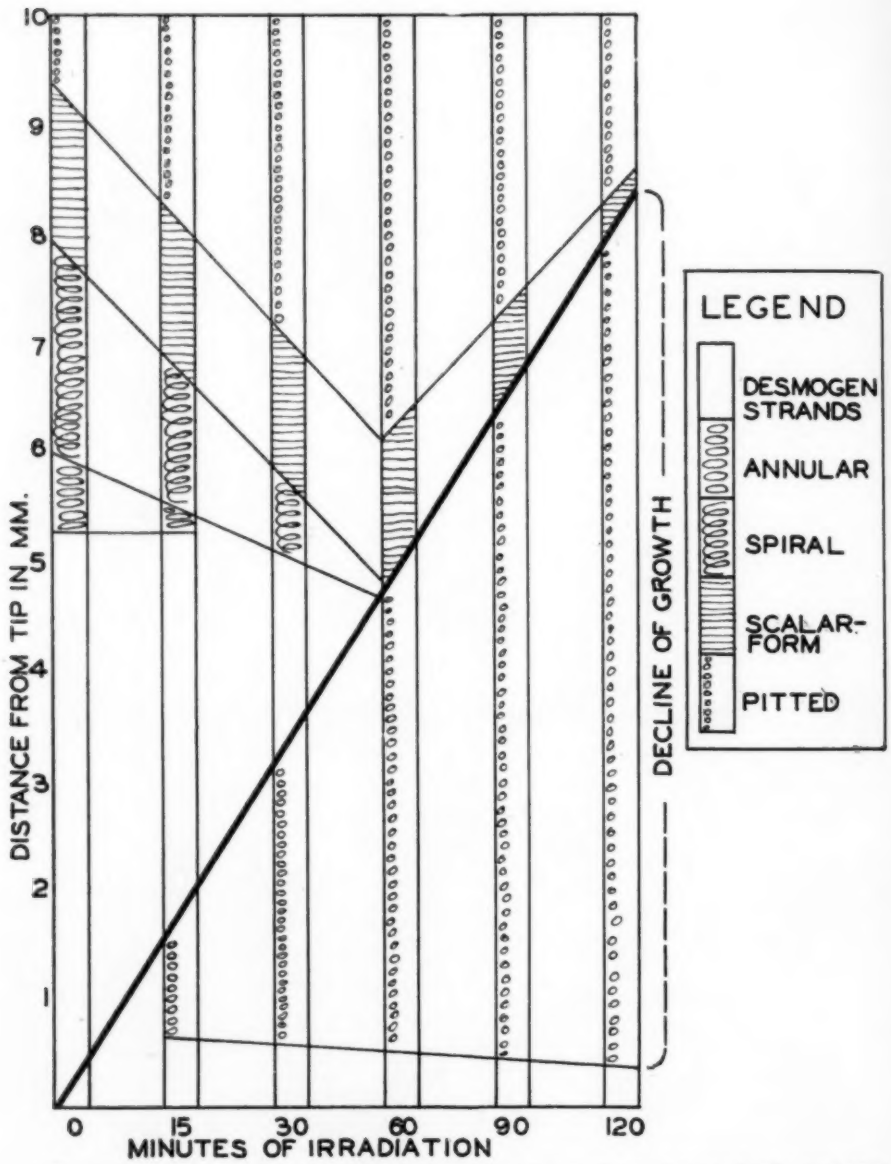

FIG. 8. Diagram showing the order and relative range of vessel types in the root tips of control *Vicia faba* seedlings and of seedlings grown from seeds x-ray irradiated at 30 kv., and 10 ma. for 15, 30, 60, 90, and 120 minutes.

total extent of elongation and the length of their germination period before subsequent decline are dependent upon the magnitude of the x-ray treatment. They are found to decrease with increasing treatments. It seems justi-

fiable to proceed further and to associate histological data with these external observations.

It would be expected that indications of decline in extent of cellular elongation in the roots would appear first in the most recently formed vessels. This is observed in all seedlings from the x-ray treated seeds; in them, portions of the root tip have become completely permanent in aspect by the increasing presence of xylem elements with pitted secondary wall thickenings. The effect is found to become more pronounced with higher x-ray treatments. It is very prominent in the root tip sections of seedlings grown from seeds irradiated for 60, 90 and 120 minutes (figs. 5, 6, 7). It seems quite possible, therefore, that there is a relationship existent between the decreasing total elongation of the apical portions of the root and the lignification of desmogen cells going on at that time.

In these roots, it is observed that the inner walls of the young desmogen cells, show an immediate pitted lining of cytoplasm (figs. 3A, 4A), which with subsequent secondary wall thickening, forms the initial pitted vessel character. The fact that this pitted wall thickening is maintained can be related to the arrested elongation of surrounding cells.

The occurrence of pitted vessels in the tips of roots of seedlings grown from x-ray treated seeds, may therefore be interpreted as indicating a period of declining growth so far as it includes elongation, and as portraying the time of initiation of this decline by the extent of these pitted elements upward from the root apice. This suggests a turning point in normal elongation during the early part of germination to a decline resulting finally in a growth cessation. In the seedlings from seeds irradiated for 120 minutes, growth cessation as recognized by the failure of a further increase in length was apparent when the material was taken for histological examination, so that in them it is possible to inspect the extent of elongation during early germination and growth decline before "delayed killing" based on the types of secondary wall thickenings of the xylem elements. Other seedlings throughout the dose range of x-rays selected represent conditions further removed from "delayed killing" as the treatment was decreased. All, however, show similar indications of a decrease in extent of cellular elongation by the presence of pitted wall thickenings in the most recently formed xylem elements. This information is diagrammed in figure 8.

The manner of elongation in the roots of the treated seedlings indicated in normal elongation and in decline of elongation is also shown in the diagram. Normal elongation is recognized by the formation of the annular, spiral, scalariform and pitted vessels, as they may be, in the upper portion of the elongation region. Growth decline, climaxing with cessation of growth. based on the failure of further increase in length, is observed by the presence of pitted vessels in the lower part of the region of root elongation. The

extent of normal growth is found to decrease, and the extent of growth decline is found to increase, with increasing x-ray treatments.

The observations are briefly summarized in the following paragraphs:

1. Normal *Vicia faba* seedlings (fig. 2). Vessel formation starts with vacuolation of desmogen cells, causing the cytoplasm to form a pitted lining on the inner cell wall (fig. 2, A). The first vessel secondary wall thickening is annular, which is followed basipetally by spiral, scalariform, and pitted formations. These are considered to be related to the extent of elongation taking place in the region of the root in which they are being formed; annular and spiral in the region of rapid elongation, and scalariform and pitted in regions of slight elongation.

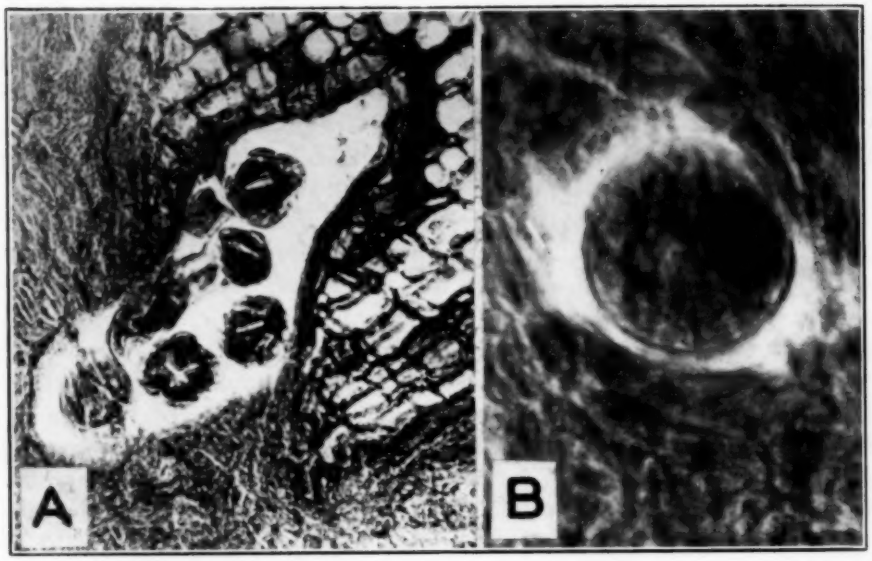

FIG. 9. Modifications about the root tip meristem in seedlings grown from seeds x-ray irradiated at 30 kv., 10 ma., for 120 minutes. A. Extensive lignification with cavities containing giant nucleus-like structures. ×450. B. Enlargement of a structure shown in A. ×about 1000.

2. Seedlings grown from seeds irradiated for 15 minutes (fig. 3). In the roots of these seedlings above the pitted vessels of the tip, a region of desmogen strands is present but is less extensive than that observed in control roots. The first vessel type is represented by a few annular elements. Spiral and scalariform thickenings are far more abundant, and are followed by a prominent region of larger pitted vessels.

3. Seedlings grown from seeds irradiated for 30 minutes (fig. 4). Above the pitted vessel present in the lowermost portion of the region of elongation, desmogen cells with vacuolated cytoplasm are evident, but these are

rapidly followed by spiral vessels with scalariform and pitted elements appearing in regions above. No annular vessels are observed.

4. Seedlings grown from seeds irradiated for 60 minutes (fig. 5). In roots of these seedlings few desmogen cells are found above the pitted vessels in the tip. Thickenings of the scalariform type appear very rapidly, and a great prominence of pitted vessels are found above. Annular and spiral elements are observed only occasionally in these roots.

5. Seedlings grown from seeds irradiated for 90 minutes (fig. 6). The region of pitted vessels is very pronounced and extends upward in these roots for approximately 6.5 mm. This is followed by a few desmogen strands which soon show wall thickenings of the scalariform type. These, however, are considerably less in number than in preceding lots. The pitted vessels appearing next are found throughout the remaining part of the region of root elongation.

6. Seedlings grown from seeds irradiated for 120 minutes (fig. 7). The vessel type in roots of these seedlings is almost completely pitted; a few scalariform elements are found approximately 8.5 mm. from the tip (fig. 8). Further inspection of these roots indicates a very extensive lignification of the tip, in which cavities are observed containing large structures which seem to represent giant nuclei. These structures are shown in figure 9, but no explanations are attempted here. The very pronounced curling of the root tip appears characteristically in seedlings irradiated with this dose of x-rays.

SUMMARY

1. Roots of *Vicia faba* seedlings grown from unsoaked seeds irradiated with soft x-rays, show a decreasing total elongation as increasing doses of x-rays are employed.

2. A histological study of such roots and of control roots affords adequate data to demonstrate experimentally that the type of wall thickening found in vessels is determined by the extent of elongation in surrounding tissues.

3. After the onset of "delayed killing" in seedlings grown from x-ray irradiated seeds, evidenced by a decline in growth as it involves elongation, only pitted vessels are formed. This is apparently related to a markedly decreased extent of elongation of cells surrounding the vessels.

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69(3): 235–239 MARCH, 1942

SOMATIC CHROMOSOMES OF ACONITUM NOVEBORACENSE AND A. UNCINATUM[1]

DOROTHY J. LONGACRE

The reduced chromosome number of *Aconitum napellus* was reported as 12 by Overton (7). Osterwalder (6), presumably working with the same type of material, also reported the haploid number for this species to be 12. Langlet (4) reported the chromosome number for a series of aconites in which he listed the diploid number as 16. Since the basic number has been established as 8, it is evident that Overton and Osterwalder were dealing with triploid aconites and not with diploid plants. Afify (1) reported *A. napellus* as a tetraploid having a chromosome number of 32. Schafer and La Cour (8) tabulated the chromosome number for the aconites of the blue-flowered group (*Eu-Aconitum*) as follows: diploids with 16 chromosomes, triploids with 24, tetraploids with 32, hexaploids with 48, and octoploids with 64. The diploid chromosome number of *A. noveboracense* was reported by Bonisteel (2) as 16.

The purpose of this study was to determine the chromosome number of the species of aconites which are indigenous to the Appalachian Mountains of the eastern United States. It was possible to obtain authenic material of only *A. noveboracense* (Gray) and *A. uncinatum* (L).

MATERIALS AND METHODS

Dormant tubers of *A. noveboracense* were collected along the Beaverkill in the Catskill Mountains in October 1940 and tubers of *A. uncinatum* were obtained from E. C. Robbins, Ashford, N. C. The dormant tubers of both species were potted and maintained in a resting condition until January when growth was resumed.

Root tips were fixed in Craf's modification of Nawaschin Fluid, F.A.A., and 2 Be of Schafer and La Cour. All of these fixatives proved to be satisfactory but most of the material was fixed with the Craf modification. Sections were cut from 10 to 15 microns thick and were stained by the iodine-gentian-violet technique and also by the iron-alum-hematoxylin methods. Warmke's modification of Belling's aceto-carmine smear method was used on root tips. Aceto-orcein (0.5 per cent in 45 per cent acetic acid) was also used. It was found that smears made with aceto-orcein were clearer than those made with aceto-carmine.

[1] The writer wishes to express her appreciation to Professor Wm. J. Bonisteel for materials used in this study and to Professor C. A. Berger, S.J., for constructive criticism and suggestions.

The metaphase plates and idiograms were outlined and partially drawn by the use of the camera lucida. For finer details of chromosome structure the smears made with aceto-orcein proved to be the most satisfactory. The photomicrographs were made with an oil immersion lens at a magnification of 450 diameters and subsequently enlarged to 1350 diameters.

OBSERVATION AND DISCUSSION

Examination of fixed and stained preparations and of root tip smears of *A. noveboracense* and *A. uncinatum* proved that the diploid chromosome number in somatic tissues is 16. This confirms the results of previous workers (2, 4, 8) that the basic number for these plants is 8. There is no evidence in well fixed preparations of any variation in the chromosome number of individuals of these species. It is assumed that the meiotic behavior of these plants is normal since they set seed freely in their native habitats. Seeds were also collected from the plants growing in the greenhouse.

In root tips of Sparks aconite, a triploid species, Bonisteel found the chromosome number of certain sectors to be twice the number of the normal somatic count. Schafer and La Cour also reported that the two diploid species, *A. transsectum* and a clone of *A. vulparia*, had tetraploid sectors present with diploid somatic tissue. The two species of plants under investigation were carefully examined for any sectors that would show an increase in the normal chromosome complement that might be due to a failure of spindle formation and the subsequent production of sectors which are of chromosomal chimeras. It is to be noted that the spontaneous origin of such sectors in normal somatic tissues might have a bearing upon the extended polyploid series of plants that occur in the normal range and distribution of the aconites.

The somatic chromosomes of *A. noveboracense* ($2n = 16$) are shown in figure 6 from an equatorial plate preparation at metaphase. The comparable chromosomes of *A. uncinatum* ($2n = 16$) are shown in figure 7.

The total chromosome length of *A. noveboracense* was found to be 94.8 microns and that of *A. uncinatum* to be 103 microns. The chromosome lengths recorded for the two species studied in this investigation agree with the findings of other workers. Schafer and La Cour calculated the total length of all chromosomes in the species that they studied and found that a difference exists between the chromosome length in the blue-flowered aconites (section *Eu-Aconitum*) and the yellow-flowered aconites (section *Lycoctonum*). They conclude from their studies that total chromosome length appears to give no clue to the systematic position of the aconites. The average chromosome length in the blue-flowered group listed by Schafer and La Cour was between 80.6 and 96.8 microns. *A. noveboracense* and *A. uncinatum* belong to the section *Eu-Aconitum*. The total chromosome length

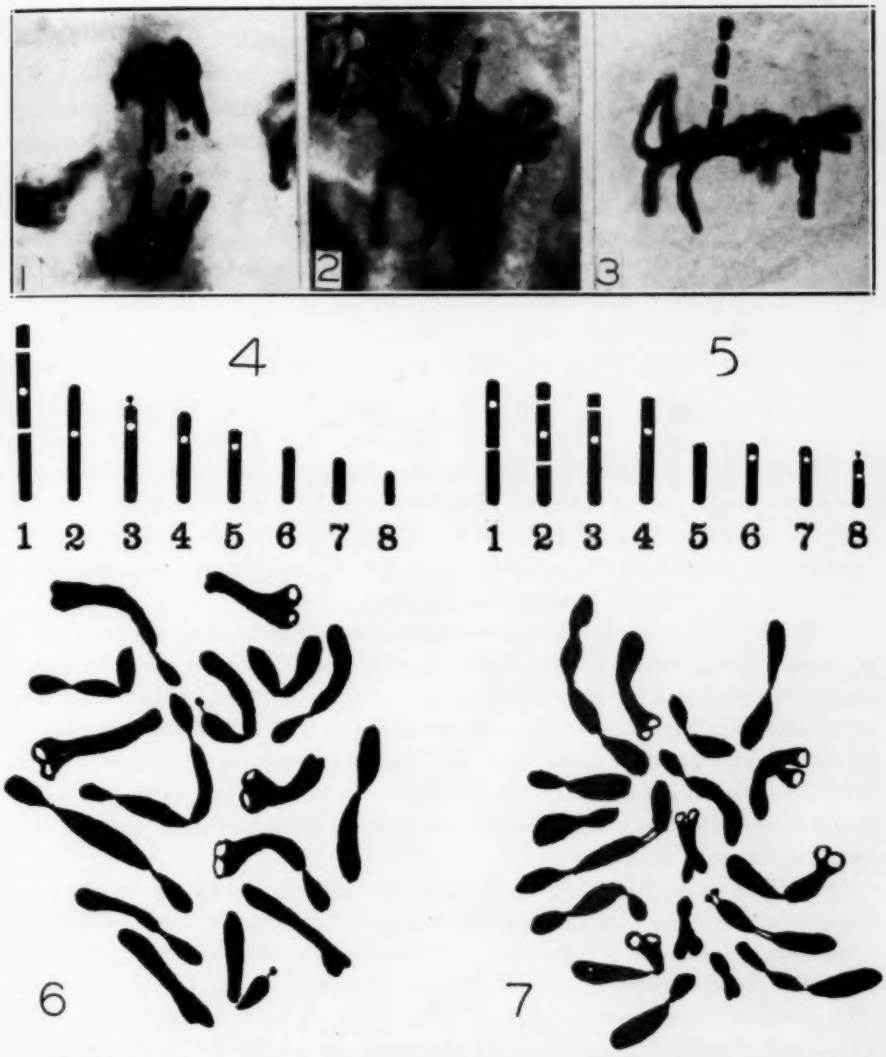

FIG. 1. *A. noveboracense*. Anaphase showing the satellites on chromosome 3. Smear method of Warmke and Belling. Aceto-orcein smear. × 1350. FIG. 2. *A. noveboracense*. Early anaphase showing one satellited chromosome and constrictions on the chromosomes that are in focus. Aceto-orcein smear method. × 1350. FIG. 3. *A. uncinatum*. Early anaphase showing constrictions at the spindle attachment region and secondary constrictions in both arms in chromosome 2. Aceto-orcein smear method. × 1350. FIG. 4. *A. noveboracense*. Idiogram with one set of chromosomes. FIG. 5. *A. uncinatum*. Idiogram with one set of chromosomes. FIG. 6. *A. noveboracense*. Metaphase plate from root tip. Fixed in Craf and stained with iodine-gentian-violet. FIG. 7. *A. uncinatum*. Metaphase plate from root tip. Fixed in Craf and stained in iron-alum-hematoxylon.

of *A. noveboracense* bears out the findings of Schafer and La Cour but that of *A. uncinatum* is somewhat greater as reported here.

When the idiogram of *A. noveboracense* (fig. 4) is compared with that of *A. uncinatum* (fig. 5) it is found that the chromosomes fall into a definite pattern in which 1, 2, 3, and 4 chromosomes from each species are characterized by their extreme length in comparison with the three or four other chromosomes of each species which are much shorter. Only one set of chromosomes is shown in figures 4 and 5. In *A. noveboracense* the chromosomes range in size from 12.9 to 1.2 microns, and in *A. uncinatum* from 9.2 to 3.2 microns. The individual chromosome lengths are given in the following table:

Chromosome designated	1	2	3	4	5	6	7	8	
A. noveboracense	12.9	7.2	6.7	6.5	5.6	4.0	3.3	1.2	microns
A. uncinatum	9.2	9.1	8.8	8.2	4.4	4.4	4.2	3.2	microns

As Longley (5) has pointed out, length measurements of chromosomes are exceedingly useful both for identification and for comparison, but they must be used with caution because of the uncertainty that chromosomes are in the same phase in different cells. Moreover, the degree of chromosome contraction varies with the kind of fixative used.

The spindle attachment region varies from terminal or subterminal to median. Darlington (3) states that *Aconitum* and *Aucuba* are exceptional plants in having long attachment constrictions in place of the short constrictions found in most plants. In chromosome 2 of *A. uncinatum* (fig. 3), as shown in smear preparations, the region of spindle attachment is extremely long. The secondary constrictions are also longer than those normally found. Darlington illustrates this type of constriction in aconite chromosome forms at mitosis.

Satellites were found in both species of aconites studied. In *A. noveboracense* one large satellite was found on chromosome 3 in figure 4 and 6. In late anaphase (fig. 1) the two satellites are shown while in figure 2 the same satellited chromosome is shown at an earlier stage. In *A. uncinatum* (fig. 5 and 7) one small satellite is illustrated on chromosome 8. Satellites were more clearly defined in smear preparations where the chromatin material was well spread.

SUMMARY

1. The somatic chromosome number of *A. noveboracense* and *A. uncinatum* is 16 ($2n = 16$).

2. Both *A. noveboracense* and *A. uncinatum* have 8 distinct types of chromosomes; four of these are long and four are short.

3. The extreme length of the secondary constrictions of *A. noveboracense* and *A. uncinatum* confirm the reports of Darlington that aconites in general are characterized by longer secondary constrictions than most plants.

4. *A. noveboracense* has one chromosome with a satellite and *A. uncinatum* has one short chromosome with a satellite.

5. The idiograms of *A. noveboracense* and *A. uncinatum* are compared as to chromosome size and shape.

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STUDIES IN THE ERICALES: A NEW NAME IN BLUEBERRIES

W. H. CAMP

It has recently been demonstrated that the low-growing, glaucous, evergreen, eglandular blueberry of the Gulf Coast is a basic, archetype diploid, and that the species (*Vaccinium myrsinites*) with which it has long been associated as a variety is an allotetraploid derived only in part from this species. While this in itself is scarcely enough to warrant recognition of both as different species, the characters which separate the two are quite as good as those traditionally accepted by more cautious students between other species of the genus. It is admitted that plants may be found, especially in western Florida, which seem to bridge the gap between *V. myrsinites* and this species. This, however, is primarily the result of a peculiar type of genic saltation within the allotetraploid *V. myrsinites* population. This phenomenon will be discussed in more detail in another place. Being preoccupied as a specific name in the genus, the name of the variety cannot be raised in rank. The following new name is therefore proposed:

Vaccinium darrowi Camp, nom. nov. *Vaccinium myrsinites* var. *glaucum* A. Gray, Synop. Fl. N. A. **2**: 21. 1878.

The species is named in honor of Dr. George M. Darrow of the United States Department of Agriculture who has been actively interested in clarifying the biological situation underlying those blueberries which are particularly promising for breeding. It is due in great part to his efforts that we have learned of the genetic situation, not only in the present species under consideration, but in other and more perplexing *Vaccinium* populations.

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A NEW CAMPANULA FROM IDAHO

ROGERS MCVAUGH

The mountainous areas of central Idaho are still relatively inaccessible and are, as a consequence, not well known botanically. It is therefore of peculiar interest that I am able to present evidence of the general occurrence in this region of a Campanula which appears to be endemic. The plant in question, so far as may be ascertained, is closely related to *Campanula parryi* A. Gray, which occurs in mountainous areas from southern Wyoming to northern New Mexico, west to southern Utah and northern Arizona. Although the known ranges of the two plants are not contiguous, the differences between them do not seem to be fundamental ones, and the Idaho plant may be designated as a variety of *C. parryi*.[1] Typical *Campanula parryi* may then be called var. *parryi*.[2]

CAMPANULA PARRYI var. IDAHOENSIS McVaugh

Plants perennial, glabrous except for the bases of the leaves of vegetative shoots and those of the lowermost leaves of the flowering stems (hypanthium sometimes pubescent). Rootstocks slender, much branched, the branches terminating in rosette-like clusters of leaves from which arise in summer, by elongation of the axes, the flowering stems. Flowering stems erect, 0.5–1.5 mm. in diameter at base, often with strongly ascending branches, few–35 cm. high, clustered from adjacent branches of the rootstock, usually one only arising from each branch. Leaves cauline and basal, few–15 on each stem, the lower sometimes approximate. Uppermost cauline leaves usually bractlike, linear or narrowly elliptic, 0.3–1.0 (2) mm. wide, 2–10 (25) mm. long, entire, blunt or acute. Middle cauline leaves linear to lanceolate, elliptic, or oblanceolate, mostly 2–5 mm. wide, 2–5 cm. long, acute or blunt at tip, somewhat narrowed or attenuate at base, entire or remotely and minutely callose-denticulate, the teeth usually not apparent to the unaided eye. Basal (rosette) leaves and lowermost cauline leaves elliptic, oblanceolate or occasionally obovate, rounded at tip, the base merely acute or attenuate and subpetiolate; petiole-like bases (at least of the rosette leaves) with stiff white cilia on the margins. Blades of these leaves 3–12 mm. wide, 7–28 mm. long, including petiolar base, usually 2–3 (5) times as long as wide, often remotely denticulate but the teeth usually not apparent to the unaided eye. Margins

[1] *Campanula parryi* A. Gray, var. **idahoensis** McVaugh, var. nov., a planta typica foliis integris, lobis calycis maturi 2–6 (raro 9) mm. longis integris, pedunculis terminalibus 0.2–1.5 (raro 5) cm. longis, corollis 9–15 mm. longis differt. Type: "Swamp Lake, on trail from Kooskooska Meadows to Friday's Pass, Selway Forest, Idaho, 7000 ft.," *J. E. Kirkwood & J. W. Severy 1638*, August 26, 1923; in the Herbarium of Montana State University, Missoula.

[2] *Campanula parryi* A. Gray, var. **parryi** McVaugh, nom. nov. *Campanula parryi* A. Gray, Syn. Fl. ed. 2, 2[1]: suppl. 395. 1886.

of all leaves usually thickened and minutely papillose-roughened. Flowers erect or slightly nodding, terminating the stems, the uppermost leaf (bract) 2–15 (rarely 50) mm. below the flower which terminates the main axis. Additional flowers, if present, 1–3, terminating the axillary branches. Hypanthium in anthesis cup-shaped, campanulate or obconic, glabrous, or sparsely or densely beset with short white flaccid hairs, 2–4 mm. long, usually slightly longer than broad when pressed, rounded or attenuate at base but usually with an abrupt break between hypanthium and peduncle. Capsule erect, campanulate, turbinate or ellipsoid, broadest at or above the middle, about 3 mm. in diameter by 7–11 mm. long, wholly inferior, attenuate or abruptly rounded at base, opening near the summit by three upcurled valves. Seeds ellipsoid-lenticular, about 1 mm. long, brown, very minutely lined. Calyx-lobes narrowly triangular or subulate, acute, entire, 1–2.5 mm. wide at base, 2–6 mm. long in anthesis, usually elongating in fruit, becoming (3) 6–9 mm. long; lobes appressed or somewhat spreading in flower and fruit. Corolla glabrous, broadly campanulate, 9–15 mm. long, the width when pressed usually equalling the length or slightly exceeding it. Lobes 3–6.5 mm. long, one-third to one-half the length of the whole corolla, ovate-deltoid, acute or apiculate, the sinuses V-shaped. Style 7–13 mm. long, straight, divided 1.5–2.5 mm., roughened distally more than half its length (5–8 mm.). Anthers glabrous, linear, straight, 4–5.5 mm. long. Filaments 1.5–2 mm. long, the expanded basal part rounded, ciliate, 1–1.5 mm. long, the linear distal part hyaline, about 0.5 mm. long.

Most of the species of the genus *Campanula* are notable for extreme variation in size of corolla, in number of flowers per plant, and in habit. Definitive characters pertaining to species differentiation often reside in the fruit only. For these reasons most herbaria contain numerous wrongly determined specimens of *Campanula* which have been identified while in flower only, or identified by means of keys based upon vegetative characters or those of the flower alone. It is thus thought desirable to present at this point a key for the separation of the species with which *Campanula parryi* may be confused. In the Rocky Mountain region, at high altitudes, there occur three species, all of which occasionally bear but a single flower; *C. uniflora* regularly produces but one flower on each stem, but it is clearly unsafe to refer a plant to this species by this character alone, as *C. parryi* and *C. rotundifolia*, especially under adverse conditions of habitat, may do the same thing.

KEY TO THE SPECIES OF CAMPANULA OF THE ROCKY MOUNTAIN REGION, SOUTH OF THE CANADIAN BORDER

1. Anthers 1.5–2.5 mm. long; flower regularly 1 only, erect; capsule erect, opening by valves near the summit ..... *C. uniflora* L.
1. Anthers 4–6.5 mm. long 2
   2. Bases of the rosette leaves and lower cauline leaves ciliate with white hairs up to 0.7 mm. long, the plants otherwise glabrous; flower regularly 1 only (or with 1–4 additional flowers on subordinate lateral branches); flower and capsule erect, the latter opening by valves near the summit ..... (*C. parryi*) 3

3. Leaves and calyx-lobes entire, the teeth, if any, scarcely visible to the unaided eye; calyx-lobes 2–6 (9) mm. long in fruit; corolla 9–15 mm. long, the principal peduncle 0.2–1.5 (rarely 5) cm. long ..... var. *idahoensis*

3. Calyx-lobes and at least some of the leaves usually with teeth plainly visible to the unaided eye; calyx-lobes (6) 10–15 mm. long in fruit; corolla (9) 15–23 mm. long, the principal peduncle usually 3–5 cm. long ..... var. *parryi*

2. Plants glabrous or hispidulous, the leaf-bases never long-ciliate but at most with hispid margins, the processes up to 0.2 mm. long; flowers regularly numerous, sometimes one only; capsule nodding, opening by valves at base; flowers erect or nodding ..... *C. rotundifolia* L.

It should be noted that I am including under *Campanula rotundifolia* all the plants which have passed as *C. petiolata* A. DC., as well as *C. macdougalii* Rydb. and *C. sacajaweana* M. E. Peck. I have examined the type specimens of the two latter, and in my judgment neither is sufficiently distinct to be set up as a distinct species.

I have seen the following material of *C. parryi* var. *idahoensis:*

IDAHO—ADAMS CO.: Pyramid Peak, Weiser N. F., *Christ 8684,* Aug. 8, 1937 (herb. of J. H. Christ). IDAHO CO.: Hoodoo Lake, 18 mi. s.e. of Powell R. S., *Christ 12807* July 21, 1941 (U. S. National Arboretum); dry mountain top near Powell R. S., R14E, T 35N, *R. J. Davis 3629,* June 29, 1941 (herb. Univ. of Idaho, Pocatello); Buffalo Hump, near Orogrande, in tundra, *Christ 11606,* July 31, 1940 (herb. Christ); Monroe Creek, trail crossing in Clearwater Forest, *Kirkwood 2002,* Aug. 26, 1924 (herb. Mont. State); near Friday's Pass, 7000 ft., *Kirkwood & Severy 1649,* Aug. 26, 1923 (herb. Mont. State); Friday's Pass, 7940 ft., *Kirkwood & Severy 1639* (herb. Mont. State); trail from Kooskooska Meadows to Friday's Pass, Swamp Lake, *Kirkwood & Severy 1637* and *1638* (Type) (both in herb. Mont. State).

MONTANA—MISSOULA CO.: Lolo Road, sunny site, hillside, *F. H. Rose 538,* July 19, 1938 (U. S. National Arboretum).

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69(3): 244–248 MARCH, 1942

NEW COMBINATIONS AND NEW NAMES IN THE UMBELLIFERAE—II[1]

MILDRED E. MATHIAS AND LINCOLN CONSTANCE

LOMATIUM Raf. Journ. Phys. **89**: 101. 1819. *Cogswellia* Spreng. in Linn. Syst. Veg. ed. Roem. & Schult. **6**: 48. 1820. *Euryptera* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 629. 1840. *Leptotaenia* Nutt. ex. Torr. & Gray, op. cit. p. 629. *Leibergia* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 575. 1896. *Cynomarathrum* Nutt. ex Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 244. 1900. *Cusickia* Jones, Contr. West. Bot. **12**: 39. 1908. *Peucedanum* and *Ferula* of American authors, not of Linn. 1753.

The genera *Lomatium* and *Leptotaenia* have been kept distinct by all authors treating the American Umbelliferae. The diagnostic differences were summarized by Coulter and Rose[2] as follows: "*Peucedanum* [to which the species of *Lomatium* were then referred] also differs in its membranous lateral wings, which are strongly nerved on the ventral face at the inner margin, in the absence of a longitudinal ridge on the commissural surface, and in the often solitary oil-tubes. In the case of those species of *Peucedanum* which have more than one oil-duct in the intervals, the decidedly membranous wings are in sharp contrast with those of *Leptotaenia*. . . ." Subsequent writers have combined the nature of the fruit wings with features of habit and size to distinguish the two assemblages of species.

The longitudinal ridge on the commissural surface of the mericarps is to be found in many if not all of the species of *Lomatium* as well as in those of *Leptotaenia*, and the wing-nervation appears to have little reliability. Solitary oil tubes are characteristic of *Leptotaenia Leibergi* Coult. & Rose, *L. minor* Rose and *L. salmoniflora* Coult. & Rose. As to habit, the species of *Leptotaenia* are usually stated to be tall, stout and caulescent, with broad leaves, whereas the species of *Lomatium* are described as less stout, low and acaulescent or nearly so, with smaller leaves. It is true that *Leptotaenia dissecta* Nutt. and its var. *multifida* (Nutt.) Jepson, *L. purpurea* (Wats.) Coult. & Rose and *L. californica* Nutt. are all tall, stout and caulescent with very broad leaves. However, *Lomatium Suksdorfii* (Wats.) Coult. & Rose and its var. *Thompsonii* Mathias and *L. triternatum* (Pursh) Raf. vars. *anomalum* (Jones) Mathias and *macrocarpum* (Coult. & Rose) Mathias are equally large, robust and caulescent, with leaves of comparable size. *Leptotaenia anomala* Coult. & Rose, *L. Bradshawii* Rose, *L. Leibergi*, *L. minor*, *L. salmoniflora* and *L. Watsonii* Coult.

[1] The first paper of this series was published in Bull. Torrey Club **68**: 121–124. 1941.
[2] Rev. N. Am. Umbel. 51. 1888.

& Rose, on the other hand, are all relatively small plants, which are either acaulescent or short-caulescent, and provided with proportionately small leaves.

The habital similarity between certain species customarily assigned to *Leptotaenia* and others retained in *Lomatium* is both striking and confusing. Thus, *Leptotaenia anomala* is scarcely distinguishable in flower from *Lomatium caruifolium* (Hook. & Arn.) Coult. & Rose and *Lomatium marginatum* (Benth.) Coult. & Rose, and all are closely related by their several, obscure oil tubes and semi-orbicular or obovate fruit. *Leptotaenia Bradshawii* is habitally very similar to *Lomatium leptocarpum* (Nutt.) Coult. & Rose, and grows in nearly identical habitats—moist swales. *Leptotaenia salmoniflora* is scarcely separable from *Lomatium Grayi* Coult. & Rose unless either fresh flowers or ripe fruit are present. *Leptotaenia Leibergi* so closely resembles such bulbous species of *Lomatium* as *L. Canbyi* Coult. & Rose that it had been previously described as *Peucedanum* (*Lomatium*) *Hendersoni* Coult. & Rose. It is clear from the above that differences in habit may permit the separation of certain species of the two genera from each other, but does not in itself render the two groups of species generically distinct.

We must necessarily turn, then, to a re-examination of the characters of the fruit, which have furnished the chief technical grounds for retaining the two genera. The typical *Leptotaenia* fruit, that found in *Leptotaenia dissecta* and var. *multifida*, *L. purpurea* and in most plants of *L. californica*, superficially resembles a pumpkin seed: the wings are very thick and corky, and form a prominent, narrow border to the mericarp. In *L. Leibergi*, *L. minor*, *L. salmoniflora* and *L. Watsoni* the dorsal surface of the mericarp and of the wing are uniformly brown in color, with the result that the only slightly thickened wing is scarcely visible unless the ventral surface or a transverse section of the mericarp is examined. The fruit of *L. anomala* is very much like that of *Lomatium caruifolium*, which has "thickish" wings, and *L. marginatum*, except that it is somewhat more corky-thickened. *Leptotaenia californica* is remarkable for the fact that fruit from the southern portion of the range has the "pumpkin seed" form, while that from the northern limits is similar, but thin-winged. The traditional generic distinction thus breaks down in this one species.

It seems evident that the single technical distinction of wing thickness has been over-emphasized to keep apart under *Leptotaenia* an unnatural group of species, the members of which show close relationships to different species of *Lomatium*. Accordingly, we find it necessary to incorporate *Leptotaenia* in the genus *Lomatium*. This change requires the following new combinations and new names, the first of which has already been indicated in our herbarium by Dr. Robert F. Hoover.

Lomatium humile (Coult. & Rose) Hoover, comb. nov. *Leptotaenia anomala* Coult. & Rose, Rev. N. Am. Umbel. 53. 1888, not *Lomatium anomalum* Jones, 1900. *Leptotaenia humilis* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 200. 1900. *Leptotaenia humilis* var. *denticulata* Jepson, Madrono **1**: 146. 1923. *Lomatium caruifolium* var. *denticulatum* Jepson, op. cit., p. 151. 1924.

Lomatium Bradshawii (Rose ex Mathias) Mathias & Constance, comb. nov. *Leptotaenia Bradshawii* Rose ex Mathias, Lflts. West. Bot. **1**: 101. 1934.

Lomatium dissectum (Nutt.) Mathias & Constance, comb. nov. *Leptotaenia dissecta* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 630. 1840. *Leptotaenia dissecta* var. *foliosa* Hook. Lond. Journ. Bot. **6**: 236. 1847. *Cynapium Bigelovii* Torr. Pacif. RR. Rept. **4**: 94. 1856. *Ferula dissecta* Gray, Proc. Am. Acad. **7**: 348. 1868. *Ferula dissoluta* Wats. in Brewer & Wats. Bot. Calif. **1**: 271. 1876. *Leptotaenia foliosa* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 198. 1900.

LOMATIUM DISSECTUM (Nutt) Mathias & Constance var. **multifidum** (Nutt.) Mathias & Constance, comb. nov. *Leptotaenia multifida* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 630. 1840. *Ferula multifida* Gray, Proc. Am. Acad. **7**: 348. 1868. *Leptotaenia Eatoni* Coult. & Rose, Rev. N. Am. Umbel. 52. 1888. *Leptotaenia multifida* var. *Eatoni* Jones, Contr. West. Bot. **12**: 40. 1908. *Leptotaenia dissecta* var. *multifida* Jepson, Madrono **1**: 145. 1923.

Lomatium columbianum Mathias & Constance, nom. nov. *Ferula purpurea* Wats. Proc. Am. Acad. **21**: 453. 1886, not *Lomatium purpureum* A. Nels. 1901. *Leptotaenia purpurea* Coult. & Rose, Rev. N. Am. Umbel. 52. 1888.

Lomatium salmoniflorum (Coult. & Rose) Mathias & Constance, comb. nov. *Leptotaenia salmoniflora* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 201. 1900.

Lomatium minus (Rose) Mathias & Constance, comb. nov. *Leptotaenia minor* Rose ex Howell, Fl. N. W. Am. **1**: 251. 1898. *Cusickia minor* Jones, Contr. West Bot. **12**: 40. 1908.

Lomatium cuspidatum Mathias & Constance, nom. nov. *Leptotaenia Watsoni* Coult. & Rose, Rev. N. Am. Umbel. 52. 1888, not *Peucedanum Watsoni* Coult. & Rose, 1888, nor *Lomatium Watsoni* Coult. & Rose, 1900.

Lomatium californicum (Nutt.) Mathias & Constance, comb. nov. *Leptotaenia californica* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 630. 1840, not *Peucedanum californicum* Nutt., 1840, nor Coult. & Rose, 1888. *Ferula californica* Gray, Proc. Am. Acad. **7**: 348. 1868. *Leptotaenia californica* var. *platycarpa* Jepson, Erythea, **1**: 8. 1893. *Leptotaenia californica* var. *dilatata* Jepson, op. cit., p. 63.

PODISTERA Wats. Proc. Am. Acad. **22**: 475. 1887. *Ligusticella* Coult. & Rose, Contr. U. S. Nat. Herb. **12**: 445. 1909. *Orumbella* Coult. & Rose, op. cit., p. 446.

In a recent paper,[3] the authors pointed out the untenability of the genus *Orumbella* and transferred its sole species to *Ligusticella*, hitherto also represented by a single species. It was surprising, therefore, to be unable to find valid generic characters to separate *Ligusticella*, thus reconstituted, from *Podistera*, a third reputedly monotypic genus. *Podistera* is based upon *P. nevadensis* (Gray) Wats., a species first questionably referred to *Cymopterus* Raf. by Gray, who had at hand only inadequate material. The senior author has already suggested[4] that *Podistera* differs markedly from *Cymopterus* in the possession of a stylopodium and in the lack of wing-development of the fruit. The compression of the fruit, moreover, is lateral in *Podistera* and dorsal in *Cymopterus*, which indicates that these genera are scarcely consanguine. On the other hand, *Podistera* agrees with *Ligusticella* in these and all other important diagnostic features. We are thus forced to combine the three supposedly monotypic genera into a single genus. The resultant genus has a remarkable distribution, with one species in Alaska, a second alpine in California, and the third alpine in Colorado. The following new combinations are needed:

Podistera Eastwoodae (Coult. & Rose) Mathias & Constance, comb. nov. *Ligusticum Eastwoodae* Rose ex Eastw. Zoe **4**: 17. 1893, nomen nudum; Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 320. *pl. 13.* 1895. *Ligusticella Eastwoodae* Coult. & Rose, Contr. U. S. Nat. Herb. **12**: 445. 1909.

Podistera Macounii (Coult. & Rose) Mathias & Constance, comb. nov. *Ligusticum Macounii* Coult. & Rose, Contr. U. S. Nat. Herb. **1**: 289. *pl. 23.* 1893. *Orumbella Macounii* Coult. & Rose, Contr. U. S. Nat. Herb. **12**: 446. 1909. *Ligusticella Macounii* Mathias & Constance, Bull. Torrey Club **68**: 123. 1941.

PRIONOSCIADIUM Wats. Proc. Am. Acad. **23**: 275. 1888. *Langlassea* Wolff ex Fedde, Repert. **9**: 420. 1911.

PRIONOSCIADIUM THAPSOIDES (DC.) Mathias var. **Pringlei** (Wats.) Mathias & Constance, comb. nov. *Prionosciadium Pringlei* Wats. Proc. Am. Acad. **23**: 276. 1888.

PTERYXIA Nutt. ex Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 170. 1900. *Cymopterus* § *Pteryxia* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 624. 1840. *Pseudopteryxia* Rydb. Bull. Torrey Club **40**: 71. 1913. *Pseudoreoxis* Rydb. op. cit., p. 73.

Three species, which have heretofore collectively found a place in *Pseudocymopterus* Coult. & Rose and have individually been referred also to *Cymopterus*, *Aletes* Coult. & Rose and *Pseudopteryxia*, have not exhibited complete conformity with any of these groups. The habit, the prominent rather than obsolete to evident calyx lobes and the membranous rather than spongy wings of the fruit relate these species more closely to

[3] Bull. Torrey Club **68**: 123. 1941.

[4] Ann. Mo. Bot. Gard. **17**: 221, 245, 248. 1930.

Pteryxia than to the type species of *Pseudocymopterus*. We believe that referring these species to *Pteryxia* will result in a more natural classification. The following transfers are required:

Pteryxia anisata (Gray) Mathias & Constance, comb. nov. *Cymopterus ? anisatus* Gray, Proc. Acad. Phila. **1863**: 63. 1864. *Pseudocymopterus anisatus* Coult. & Rose, Rev. N. Am. Umbel. 75. 1888. *Pseudocymopterus aletifolius* Rydb. Bull. Torrey Club **31**: 574. 1904. *Pseudopteryxia anisata* Rydb. Bull. Torrey Club **40**: 71. 1913. *Pseudopteryxia aletifolia* Rydb. op. cit., p. 72.

Pteryxia Hendersoni (Coult. & Rose) Mathias & Constance, comb. nov. *Pseudocymopterus Hendersoni* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 190. 1900. *Pseudopteryxia longiloba* Rydb. Bull. Torrey Club **40**: 72. 1913. *Pseudopteryxia Hendersoni* Rydb. Fl. Rocky Mts. 624, 1064. 1917; ed. 2. 624. 1922. *Pseudocymopterus anisatus* var. *longilobus* Tidestrom, Contr. U. S. Nat. Herb. **25**: 399. 1925. (Fl. Utah & Nevada).

Pteryxia Davidsoni (Coult. & Rose) Mathias & Constance, comb. nov. *Aletes ? Davidsoni* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 107. 1900. *Pseudocymopterus filicinus* Wooton & Standl. Contr. U. S. Nat. Herb. **16**: 158. 1913. *Pseudocymopterus Davidsoni* Mathias, Ann. Mo. Bot. Gard. **17**: 282, 316. 1930.

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INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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PLANT TAXONOMY AND FLORISTICS
(exclusive of fungi)

(See also under Morphology: Williams; Withner. Under Genetics: Baldwin & Culp; Hubricht & Anderson)

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(including anatomy, and cytology in part)

(See also under Taxonomy: Fasolo; Hollingshead. Under Mycology: Johnson & Brown. Under Genetics: Coleman)

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(See also under Mycology: Timonin)

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